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Quantitative Histological Studies on the Mammary Gland of Rats during Pregnancy, Lactation and Involution

Chung Gil Lee, D.V.M., M.V.Sc., M.Sc.

Department of Tropical Veterinary Science, James Cook University of North Queensland Townsville, Q' ld. 4811, Australia

Introduction

Normal functioning of the mammary gland is clearly essential for survival of the newborn. Possibly more than any other organ, this gland is subjected to continuous and repeated changes in structure[7]. Satisfactory interpretation of morphological findings in the mammary gland necessitates a clear understanding of these structural changes, particularly at the histological level. Morphology and composition of the mammary gland are dependent upon the physiological condition of the animal. Therefore, quantitation of mammary composition is difficult. However, many attempts have been made by employing histological[5,6], biochemical[14] and autoradiographic[15] methods.

There are a number of histological methods which are basically planimetric methods.[8,11], The method of choice is based upon the principle of point counting.[5,18] A grid composed of a number of regularly dispersed points is cast at random onto the tissue to be assessed. The number of points falling on any component of the tissue is proportional to its volume.

In the present study, quantitative histological changes in the mammary gland of rats during pregnancy, lactation and involution were studied using a point counting technique.

Materials and Methods

Animals: Virgin female Wistar rats were mated at 12~15 weeks-of-age. Pregnancy was determined by observation of spermatozoa in vaginal smears; the day following the appearance of spermatozoa was counted as day 0 of gestation. Average gestation length was 22 days. The uteri of test animals sacrificed at intervals before parturition were examined to confirm pregnancy. In order to obtain uniform development of the mammary gland each litter size was adjusted to 6~8 rat pups per dam, and rats were weaned at 20 days-of-age.

Preparation of Tissues : Rats were killed at 2, 6, 10, 14 or 18 days pre-partum, 0, 4, 8, 12 or 16 days of lactation and 1, 5 or 10 days post-weaning, by exsanguination under ether anaesthesia. A midline incision was made and a secretion sample or, piece of mammary tissue was placed onto 10% sheep blood agar and incubated aerobically for 24 to 48 hours at 37°C. Tissues from which bacteria were isolated or which showed inflammation histologically were excluded from the study. A piece of tissue from right-hand side inguinal glands was fixed in formol alcohol, and sections cut at 6μm were stained with haematoxylin and eosin (H & E).

Quantitative Evaluation : Detailed quantitative histological observations were made on sections stained with H & E at 400 magnifications. Criteria were evaluated using a Weibel graticule (Wild Heerbrugg, Switzerland). Volumetric determinations were made on 20 graticule fields and the applicability was checked as described by Schaefer[12]. The components determined were: Adipose tissue: intra and interlobular connective tissue (stroma) including blood

Table 1. Changes in Mean Volumetric Proportions of Major Components during Pregnancy, Lactation and Involution of Mammary Gland of Rats (volume% of each component per 20 graticule fields)

Stage (Days)	No. of Animals	Fat	Stroma	Epithelium
Pregnancy				
2	2	87.6	8.0	4.4
6	2	76.5	14.0	9.4
10	2	77.6	12.4	9.9
14	2	62.7	14.5	22.8
18	2	62.7	10.9	26.4
Lactation				
0	4	11.5	25.6	62.9
4	3	6.9	30.9	62.2
8	3	11.7	23.0	65.3
12	3	11.1	26.6	62.3
16	3	3.9	25.5	70.6
Involution				
1	3	11.0	23.3	65.7
5	3	64.1	16.6	19.3
10	3	68.6	19.8	11.5

vessels: epithelium of the alveoli and ducts: alveolar and ductal lumen. For meaningful comparison of the three tissue components-fat, stroma and epithelium the volumetric proportions of alveolar and ductal lumens (with their contained secretions) were estimated and then disregarded. Subsequent reference to mean volumetric proportion (MVP) therefore assumes that fat, stroma and epithelium comprise 100% of udder volume.

Statistical Analysis: Mean volumetric proportions of three components were analysed by one-way analysis of variance using a PDP-10 computer. The analysis was performed separately on each stage.

Results

Changes in the MVP of major mammary components during pregnancy, lactation and involution of the mammary gland of rats are shown in Tables 1 and 2.

Table 2. Analysis of Variance for Major Components during Pregnancy, Lactation and Involution

	Sources of Variation	Degrees of Freedom	Mean Square	F
Pregnancy				
Fat	Days	4	229.6	7.83**
	Error	5	29.3	
Stroma	Days	4	55.5	1.36
	Error	5	50.9	
Epithelium	Days	4	716.7	27.85**
	Error	5	32.2	
Lacation				
Fat	Days	4	37.7	3.46*
	Error	11	0.9	
Stroma	Days	4	25.2	0.81
	Error	11	135.1	
Epithelium	Days	4	38.5	1.99
	Error	11	19.4	
Involution				
Fat	Days	2	3078.0	204.43***
	Error	6	15.1	
Stroma	Days	2	33.5	1.11
	Error	6	30.0	
Epithelium	Days	2	2571.7	73.08***
	Error	6	35.2	

* $p<0.05$; ** <0.01; *** $p<0.001$.

During Pregnancy: During early pregnancy the gland was composed mainly of fatty tissue: the rudimentary ducts were observed in the adipose tissue. The MVP of fat decreased with advancing pregnancy due mainly to the rapid increase in the MVP of epitheium, especially from gestational day 14. At day 18 pregnancy the clusters of secretory alveoli developed at the ends of the ducts and increased multiplication was evidenced by numerous mitoses in the secretory epithelium. The MVP of stroma did not show any trend during pregnancy.

During Lactation: At parturition alveoli were distended with secretion. The decrease in the MVP of fat continued until day 4 of lactation: the MVP of fat was approximately eight times lower during lactation than during pregnancy. The MVP of

stroma was higher (2.2 times) during lactation than during pregnancy, and the MVP of epithelium was 4.4 times higher during lactation than during pregnancy. No trend was established in the MVP of stroma and epithelium between the days during lactation.

During Involution: The characteristics of the gland at post-weaning day 1 were distension of alveoli and interstitial oedema due to milk stasis. The MVP of fat increased dramatically with advancing involution, and at post-weaning day 10 it reached the level during pregnancy. The slight decrease in the MVP of stroma was not significant. The MVP of epithelium decreased markedly with advancing involution. The mammary gland at post-weaning day 10 was similar to the gland at day 10 of gestation.

Discussion

The histology of the mammary gland during early pregnancy was comparable with other findings, in which the gland in rodents is composed mainly of adipose tissue.[3] The results presented in the present study also confirmed previous findings in the histological changes of the mammary gland. Mammary growth continues at a fairly constant and rapid rate throughout pregnancy,[4,17] and most of the growth is due to the epithelial components.[9] The same pattern was observed in the present study, and the marked difference in the MVP of epithelium between gestational day 18 and parturition indicate that the epithelial proliferation continued until parturition.

The previous studies in the rat and mouse[6,7] showed that the hyperplasia of epithelial tissue in the mammary gland continued during early lactation. Baldwin,[1] however, stated that very little additional development of glandular structures was evident histologically. No trend was established in the MVP of epithelium between the days during lactation in the present experiments. Of the total mammary development in normal rats, approximately 11% occurs before pregnancy, 41% occurs during pregnancy, and the remainder occurs during lactation.[16]

The results in this study showed that the epithelial development reached 43% level by gestational day 18 when compared to parturition.

The initial stages of involution involves a build up of milk pressure,[10] and during involution the processes from puberty to lactation are reversed.[2] At day 1 of involution, alveoli were markedly distended, and the MVP of three mammary components at post-weaning day 10 were approximately same as those at day 10 of gestation.

Significant component changes during pregnancy and involution were in relation to fat and epithelium but not stroma; this observation supported a statement by Schmidt *et al*[13] in regard to the goat that secretory tissue replaces fat rather than stroma.

Conclusion

Quantitative histological changes in the mammary gland of rats during pregnancy, lactation and involution were studied. The epithelial development reached 42% level by gestational day 18 when compared to parturition. The mean volumetric proportions of fat, stroma and epithelium at post-weaning day 10 were approximately same as those at day 10 of gestation. Significant component changes during pregnancy, lactation and involution were in relation to fat and epithelium but not stroma.

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쥐 乳腺의 姙娠, 泌乳 및 休止期의 組織計量學的硏究

李 政 吉

濠洲 제임스 쿠크 大學校 熱帶獸醫學敎室

抄 錄

쥐 乳腺의 姙娠, 泌乳 및 休止期에 있어서 組織計量學的變化는 다음과 같았다. 乳腺上皮의 發達은 姙娠 18日에 있어서 分娩時(100%로 했을때)에 比하여 42%에 達하였다. 離乳後 10日에 있어서 乳腺의 脂肪, 基質 및 上皮의 量的인 比率은 姙娠 10日에 있어서와 비슷하였다. 姙娠, 泌乳 및 休止期中의 主要 構成成分의 變化는 脂肪 및 上皮에 있었고 基質은 관계치 않았다.