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Histomorphological comparative study of the pregnant and lactating mammary gland in endogenous rabbits (*Oryctolagus Cuniculus*)

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Abstract

Background: comparative study of the pregnant and lactating mammary gland in local rabbits.

Objective: histological description of mammary gland of two periods (pregnant and lactating mammary gland).

Materials and methods: To determine the differences that could take place in the mammary gland between two periods of the life (pregnant and lactating) of endogenous rabbits (*Oryctolagus Cuniculus*).

Results: Grossly the mammary glands shown in rabbits (pregnant and lactating) were four or five pairs, located at the ventral surface of the thorax, abdomen, and inguinal. The mammary gland of the doe has 8 or 10 nipples. Histological results of pregnant group females contained the same histological components but there were alveoli intralobular connective tissue interlobular connective tissue, glandular duct, and adipose tissue, the alveoli lining with cuboidal cell, the ducts lining with a double layer of the cuboidal cell around by collagen fibers and fatty vacuoles the results of this study revealed that the pregnancy period showed the most development, especially in the glandular units (alveoli) because of the effect of estrogen and progesterone hormones rule in the growth of duct and alveoli and stope immediately after parturition after a drop of the placenta also secretion colostrum detection. In lactating group rabbits, the lobules were large collecting ducts lined by multiple layers of the columnar and cuboidal cells and mammary gland lobule. A magnified section of glandular alveoli showed it lined with low cuboidal secretory cells and their lumen contained secretory product and glandular alveoli lined with low cuboidal secretory units and their lumen contained secretory product. The alveoli had large lumens and were lined with cuboidal epithelium and flattened nuclei of myoepithelial cells and flattened nuclei of myoepithelial cells, the lobules have the most alveoli were large. The interlobular connective tissue that surrounded the large lobules with blood vessels and wide trabeculae of dense irregular connective tissue with adipose tissue, the lobules of a different size, and interlobular duct filled with the secretion of the trabeculae of dense irregular connective tissue the interlobular Connective tissue and adipose tissue, the lobules in a different size. The interlobular duct with secretion, intralobular duct, most alveoli were large and without secretion may be at the end of the lactation period.

Conclusion: During mammogenesis, the glandular tissue of the mammary gland was studied, it has been shown that parenchyma tissue with the pregnancy onset is increased and achieves its extreme value by the 25-30 days of pregnancy.

Keywords: Rabbits, mammary gland, pregnant rabbits, lactation and involution.

Introduction

The mammary gland is an exocrine gland that is found in all mammals that manufacture milk for young offspring nutrition. The glands are fixed in males and females through the prenatal stage, forming just a rudimentary duct tree at childbirth. During this period, mammary gland development relies on maternal hormones, (1). The canal of the mammary gland elongates postnatally into the mammary fat pad via the pubertal period.

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In pregnancy, a high concentration of serum progesterone still very signing through its receptor is constantly stimulated (2). During lactation, secretory action begins is the fall in progesterone levels next the placenta is delivered. Discharge of milk from the breast because of the tightening of the myoepithelial cells encircling the alveoli until the end of weaning when milk production is stop required. (3, 4). The making of milk to nourish offspring is lactation, which is accomplished via the mammary gland, which is postnatally growing and is very active (5). The mammary epithelium fully changes into milk- a manufacturing factory after parturition, the alveoli extend and the stromal adipocytes of the mammary gland start space retiring, by changing epithelial size several times (5). After the production of milk is stopped essential, the epithelial cells of milk-producing are exiled, besides the gland is changed into a prepregnancy formal, involution is the term of this operation, which contains extracellular matrix transformation, cataloged epithelium cell death, and adipocytes re-differentiation (6,7,8,5). Through the completion of involution, the gland remains prepared for one more cycle of pregnancy-connected growth, lactation, and consequent involution, the mammary gland can be transformed during the generative lifespan. Mammary epithelium works through these variations, its design is bi-layered with lumen fronting luminal cells and basally located myoepithelial cells, which are vital to the organ for the suitable obligation (9,5).

Material and methods

The study was used on clinically healthy ten rabbits divided into two groups the first group was five pregnant rabbits at the third stage of pregnancy, the second group was five lactating rabbits, were brought from local farms at alradhwanya and were caged in the animal house until the time of euthanasia and dissection to get all mammary glands. Each selected animal was firstly euthanized by using ketamine and xylazine and after that animal was dissected on a dissecting stage after shaving the chest area and starting to measure the rabbit weight, gland weight by the balance, teat length was taken from the base to the apex of the teats, teat diameter by using a ruler and also the distance between every two glands vertically and distance between gland horizontally and after dissected subsequently mammary glands and measure the weight of mammary gland, all the samples were collected then fixed with 10% neutral buffered formalin. Representative specimens were cut from each gland which was, firstly kept on 10% neutral buffered formalin for72 hours of the histological approach, Secondly, each specimen was dehydrated through different concentrations of ethyl alcohol(70%,80%,90%, and 100%) each for 2houres and after dehydration. third cleared with xylene for 1/2 hours. Fourth specimens were infiltrated with liquid paraffin wax on 56-58C. Fifth specimens are embedded on the paraffin wax to form blocks of paraffin. The sixth sectioned by a rotary microtome section was 5-6Mm thickness. Five strains were used for staining the sections by using stains which were: Harries hematoxylin and eosin (H&E), Verhoffes stain, Masson's Trichrome Stain**,** and Van-Giessen Stain, The histochemical examination was then done by using PAS stain Periodic acid Schiff. Micrometer calibrated to a light microscope at 10 random locations in different sections to provide mean ± SE values. Means were compared by-T test (SPSS Statistics-17.0) $P < 0.05$ (10).

Results

The present study of five pregnant does reveal that the rabbit has four or five pairs of mammary glands which was 2 pairs in the thoracic region, 2 or three pairs in the abdominal region, and 2 pairs in the inguinal region, so it is called thoracic, abdominal and inguinal mammary gland (Figure 1). The mammary glands measurements according to the site of teat were first, the thoracic region teat revealed that the distance between two mammary glands vertically Table .1 was first, the thoracic region teat revealed that the distance between two mammary glands vertically was 7.60 ± 0.24, the distance

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between gland horizontally was 3.98 ± 0.03, teat diameter was 2.96 ± 0.18, teat length was 3.00 ± 0.03, the mean of the rabbits' weight was 3.22 ± 0.03, the mammary gland weight was 1.04 ± 0.09, while the relative weight to bodyweight 32.2. Second teat pairs were in the abdominal region which revealed that the distance between two mammary glands was vertically 7.22 ±0.13, the distance between glands horizontally was 6.90 ± 0.55, teat diameter was 2.44 ± 0.21, the teat length was 2.84 ± 0.10, the mean of the rabbits' weight was 3.36 ± 0.06, the mammary gland weight was 1.96 ± 0.20, while the relative weight to bodyweight 58.33. The third teat pairs were the inguinal region which revealed that the distance between two mammary glands vertically was 8.06 ± 0.04, the distance between gland horizontally was 5.04 ± 0.05, teat diameter was 2.74 ± 0.26, the teat length was 2.88 ± 0.09(Figure 9), the mean of the rabbits' weight was 3.31 ± 0.03, mammary gland weight was 1.28 ±0.05, while the relative weight to bodyweight 38.67. Histological results of the mammary gland in the late pregnancy stage group showed the gland covered by a capsule of dense irregular connective tissue, the developing lobules became larger, the lobule, separated by trabeculae also presence of several differentiated alveoli which lined by simple cuboidal epithelium and with or without secretion (Figure3) and the intralobular connective tissue, interlobular connective tissue. , scattered around the glandular duct and there is a large amount of adipose tissue, and interlobular excretory duct with secretion lined by cuboidal epithelia, adipose tissue, and skeletal muscle (Figure 4,5) The magnified histological section of alveoli shows myoepithelial cells which play important role in contraction and is surrounded by adipose tissue (Figure 6) the alveoli lined with cuboidal cell, and the collecting ducts lining with a double layer of cuboidal cells around surround by collagen fibers and fatty vacuoles(Figure 7,9,10), the gland lobules consist of alveoli have different sizes and shapes (Figure 8) The differences between the average values of all groups in all tables were statistically significant ($p \leq 0.5$). Table3. The present study of five lactating does reveal that the rabbit has four or five pairs of mammary glands which were 2 pairs in the thoracic region, 2 or three pairs in the abdominal region, and 2 pairs in the inguinal region, so it is called thoracic, abdominal and inguinal mammary gland. In the lactating group (Figure 2). The mammary glands measurements according to the site of teat were first, the thoracic region teat revealed that the distance between two mammary glands vertically was 7.46 ±0.02, the distance between gland horizontally was 4.06 ± 0.04, teat diameter was 4.19 ± 0.02 (Figure 11), teat length was 5.22 ± 0.09, the means of the rabbits' weight was 2.44 ± 0.04, mammary gland weight was 1.55 ±0.02, while the relative weight to bodyweight 63.52. Second teat pairs were in the abdominal region which revealed that the distance between two mammary glands was vertically 5.06 ± 0.04, the distance between glands horizontally was 7.42 ± 0.05, teat diameter was 4.29 ± 0.02, teat length was 5.04 ± 0.05, the means of the rabbits' weight was 2.42 ± 0.03, the mammary gland weight was 1.32 ± 0.03, while the relative weight to bodyweight 54.09. Third teat pairs were in the inguinal region which revealed that the distance between two mammary glands was vertically 7.06 ± 0.08, the distance between glands horizontally was 5.02 ± 0.04 and the means of teat diameter was 4.20 ± 0.03, teat length was 4.96 ± 0.05, the means of the rabbits' weight was 2.46 ± 0.02, the mammary gland weight was 1.48 ± 0.02, while the relative weight to bodyweight 60.16. The differences between the average values of all groups in all tables were statistically significant in ($p \leq 0.5$) Table.3.). The Histological Section of the mammary gland in lactating stage shows lobules of different sizes. The interlobular duct with secretion, intralobular duct (Figure11), Histological Section of the mammary gland in lactating stage showing interlobular duct with secretion surrounding by interlobular c.t lining with a multilayer of cuboidal cell and adipose tissue (Figure12), Histological Section of the teat structure at the base of the teat in lactating female showing the lumen of teat canal lining

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with keratinized stratified squamous epithelia, teat sinus dense irregular connective tissue in lamina properia(Figure 13). The histomorphometric parameters of the mammary gland in the lactating stage showed that the mean values of the diameter of the lobule were 1922±0.44, the diameter of alveoli was 231 ±0.38, the diameter of Lumen of alveoli was 174±0.14, a diameter of Lumen of the intralobular duct was 301±0.13, the diameter of the lumen of the interlobular duct was 770±0.334. The mean values of several alveoli per microscopic field were 200±0.23, the epithelial height of alveoli was12.5±0.29, the epithelial height of the intralobular duct was 33.2±0.15, the epithelial height of the interlobular duct was42±0.21. The statistical analysis revealed significant differences at the level of (P<0.05) between the groups (Table 2,3).

Table (1): The Morphometric parameters of teats of pregnant doe according to the site of the teat.

Pregnant	Distance Between teat vertical Mean ±SE	Distance Between teat horizontal Mean ±SE	Diameter Of teat/mm Mean ±SE	Length of teat/mm Mean ±SE	Gland weight/kg Mean ±SE	Bodyweight/kg Mean ±SE	Relative weight/kg Mean ±SE
Thoracic Gland	7.60 ± 0.24 B	3.98 ± 0.03 C	2.96 ± 0.18 A	3.00 ± 0.03 A	1.04 ± 0.09 B	3.22 ± 0.03 A	32.2
Abdominal Gland	7.22 ±0.13 B	6.90 ± 0.55 A	2.44 ± 0.21 A	2.84 ± 0.10 A	1.96 ± 0.20 A	3.36 ± 0.06 A	58.33
Inguinal Gland	8.06 ± 0.04 A	5.04 ± 0.05 B	2.74 ± 0.26 A	± 0.09 A	1.28 ±0.05 B	3.31 ± 0.03 A	38.67
LSD	0.5025	0.9966	0.6969	0.2578	0.4202	0.1509	

The number of pregnant groups = 5 animals. SE = Stander Error. Means with a different letter in the same column are significantly different (P<0.05)

Table (2): The Morphometric parameters of teat lactating doe according to the site of the teat.

Lactating	Distance Between teat vertical Mean ±SE	Distance Between teat horizontal Mean ±SE	Diameter Of teat/mm Mean ±SE	Length of teat/mm Mean ±SE	Gland weight/kg Mean ±SE	Bodyweight/kg Mean ±SE	Relative weight/kg
Thoracic Gland	7.46 ±0.02 A	4.06 ± 0.04 C	4.19 ± 0.02 B	5.22 ± 0.09 A	1.55 ±0.02 A	2.44 ± 0.04 A	63.52
Abdominal Gland	5.06 ± 0.04 C	7.42 ± 0.05 A	4.29 ± 0.02 A	5.04 ± 0.05 B	1.32 ± 0.03 B	2.42 ± 0.03 A	54.09
Inguinal Gland	7.06 ± 0.08 B	5.02 ± 0.04 B	4.20 ± 0.03 B	4.96 ± 0.05 B	1.48 ± 0.02 A	2.46 ± 0.02 A	60.16
LSD	0.1067	0.1509	0.0871	0.2134	0.0871	0.1067	

Number of lactating groups = 5 animals. SE = Stander Error. Means with a different letter in the same column significantly different (P<0.05)

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Table (3): Micro morphometric parameters of the mammary gland in adult, Pregnant and lactating rabbit females

Parameters (µm) (Mean±SE)	Pregnant period (Mean±SE)	Lactating period (Mean±SE)
number of alveoli per microscopic field	134±0.24 *	200±0.23 *
Epithelial height of alveoli	10.7±0.11 *	12.5±0.29 *
Epithelial height of the intralobular duct	23.8±0.14 *	33.2±0.15 *
Epithelial height of the interlobular duct	32±0.19 *	42±0.21 *

M±SE=mean+ standard error. (*) represent significant differences at level (P<0.05) between groups

Figure (1): The pregnant rabbit with four pairs of teats

Figure (2): The lactating rabbit with five pairs of teats

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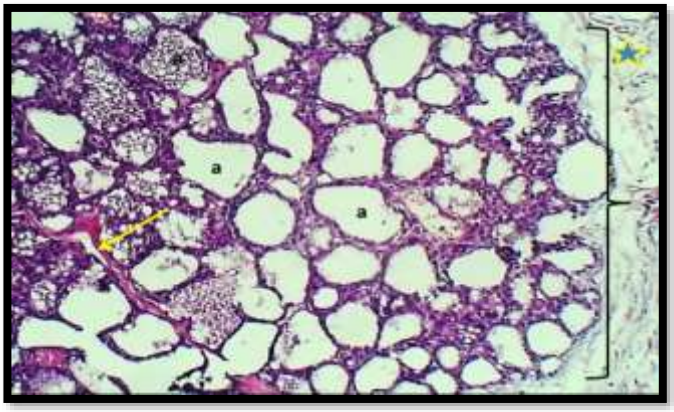

Figure(3): Histological Section of the mammary gland in late pregnancy stage showing the lobule, capsule of dense irregular connective tissue (blue star), alveoli with and without secretion(a), and Trabeculae (yellow arrow). H& E stain x40

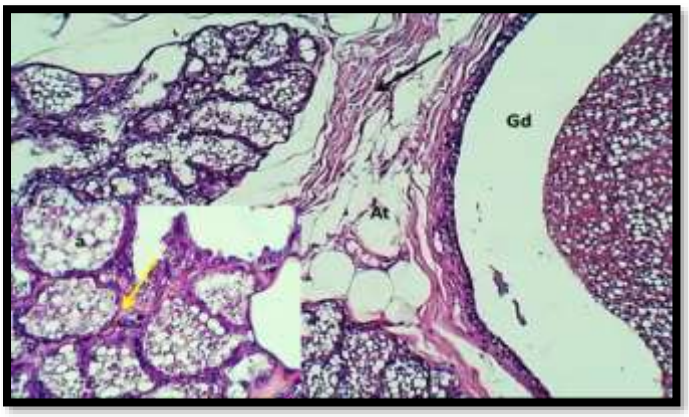

Figure (4): Histological Section of the mammary gland in late pregnancy stage showing the alveoli (a) intralobular C.T (yellow arrow) interlobular C.T. (black arrow), glandular duct (Gd), and adipose tissue (At). H& E stain x40, x100

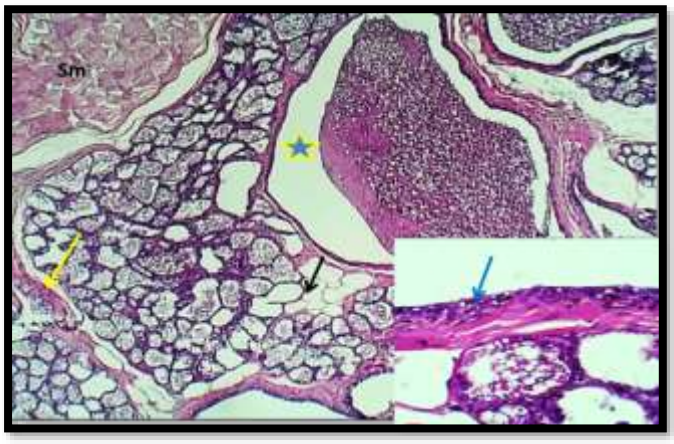

Figure (5): Histological Section of the mammary gland in late pregnancy stage showing the alveoli interlobular C.T. (yellow arrow), interlobular excretory duct with secretion (blue star) lined by cuboidal epithelial(blue arrow), adipose tissue(black arrow), and skeletal muscle(sm). H& E stain x40, x100

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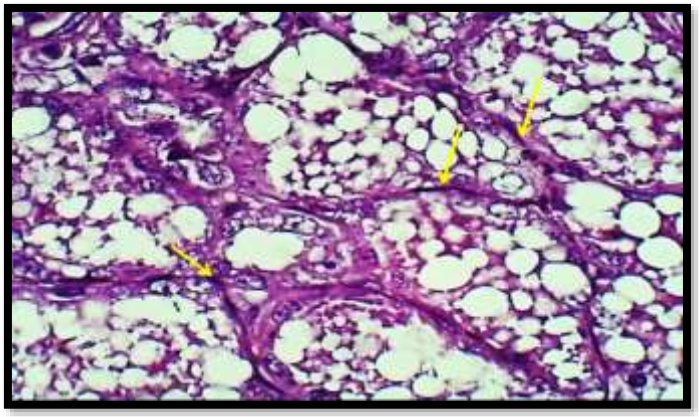
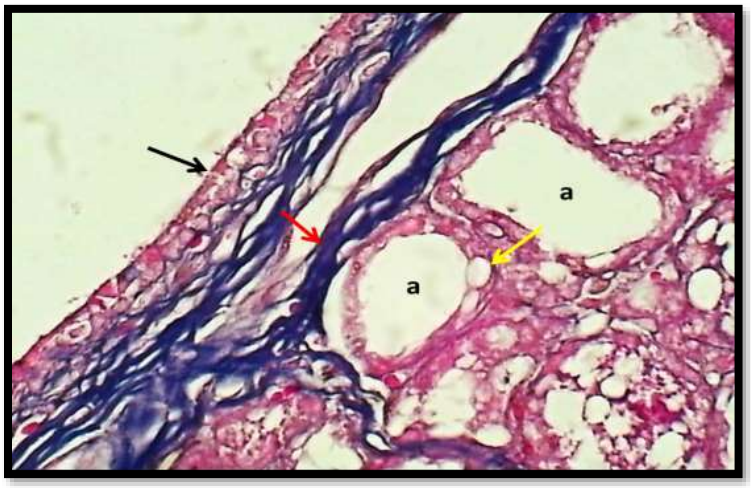


Figure (6): magnified section of alveoli showing myoepithelial cells (yellow arrows) with adipose tissue. H& E stain x400

Figure (7): Histological Section of the mammary gland in late pregnancy stage showing the lobule, dense irregular connective tissue (blue star), Trabeculae (black arrow) interlobular collagen fiber (red arrow), and alveoli(yellow arrow) Masson's Trichrome stain x40



Figure(8): magnified Section of the mammary gland in late pregnancy stage showing the alveoli lining with cuboidal cell (a), the ducts lining with a double layer of cuboidal cells (black arrows)around by collagen fibers(red arrow) and fatty vacuoles(yellow arrow),. Masson's Trichrome stain x400.

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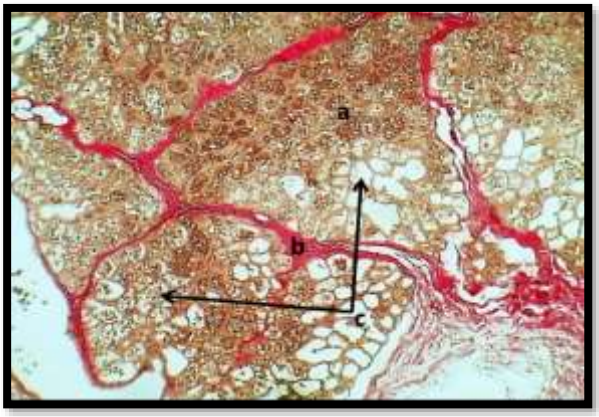
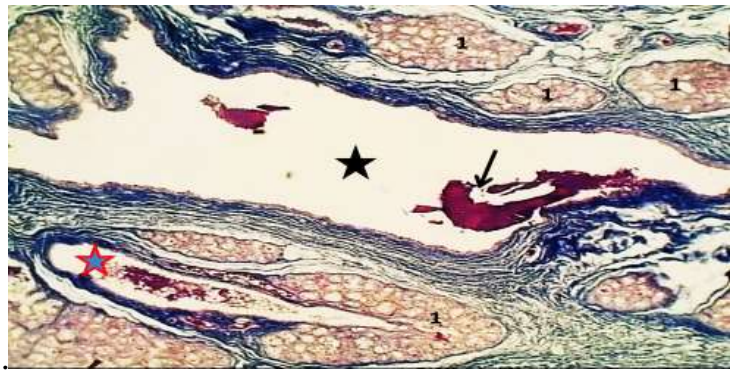


Figure (9): The Histological Section of the mammary gland in the late pregnancy stage shows the alveoli have different sizes and shapes (black arrow).The interlobular connective tissue (b) that surrounded the large lobules (a). Van Gieson's stain (x40)

Figure(10): Histological Section of the mammary gland in late pregnancy stage showing the collecting duct(blue star)lining by multiple layers of cuboidal cell(blue arrow), interlobular connective tissue (yellow arrow), and adipose tissue (black arrow). Van Gieson's stain (x40) (A).x400(B)



Figure(11):The Histological Section of the mammary gland in lactating stage shows lobules of different sizes (1).The interlobular duct (black star) with secretion (black arrow), intralobular duct (red star). Masson's trichrome stain x4

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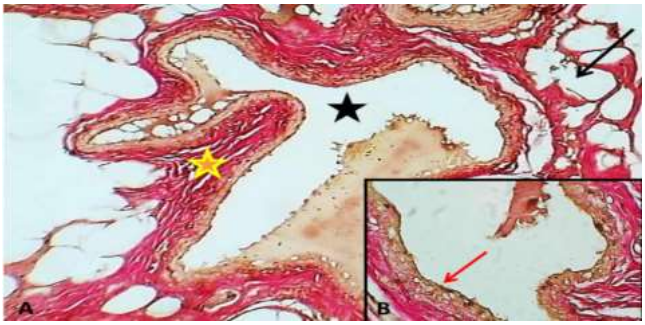

Figure (12): Histological Section of mammary gland in lactating stage showing interlobular duct with secretion (black star) surrounding by interlobular c.t.(yellow star) lining with multilayer of cuboidal cell(red arrow) and adipose tissue (black arrow) Van Gieson's stain x100(A)x400(B)

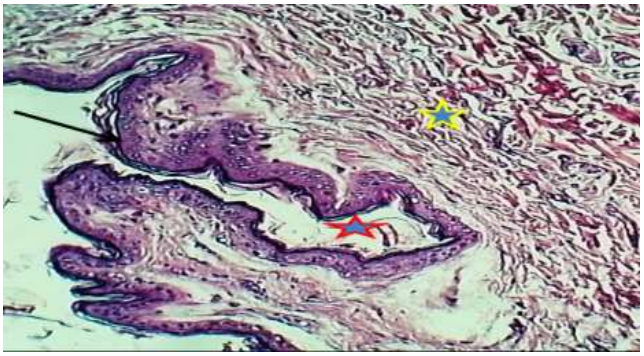

Figure (13): Histological Section of the teat structure at the base of teat in lactating female showing the lumen of teat canal lining with keratinized stratified squamous epithelia (black arrow), teat sinus (red star),dens irregular connective tissue in lamina properia (blue star). H& E stain x100

Discussion

The histological results of the mammary gland at the late stage of pregnancy show the lobules became larger due to the increase of intralobular connective tissue. The adipose tissue was few due to a large number of trabeculae of connective tissue. The growth of the ducts was continuous to form more new alveoli. This observation is similar to the reports of (11, 12) which stated that the mammary gland of bovines is composed of a widely divided ductal network with a distinct basement membrane and engraft in a stromal part. Histological results of the mammary gland in the lactating stage show the alveoli had large lumens and were lined with flattened or cuboidal epithelium. the collecting duct lining by multiple layers of the columnar and cuboidal cell, the lobules were large with interalveolar connective tissue, strands of interlobular connective tissue separated the lobules extended from a thin capsule of dense irregular connective tissue, these findings are similar to (13) who found that the buffalo epithelium of intralobular canals was lined by stratified cuboidal epithelium.

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Conclusion

During mammogenesis, the glandular tissue of the mammary gland was studied, it has been shown that parenchyma tissue with the pregnancy onset is increased and achieves its extreme value by the 25-30 days of pregnancy**.** The process of involution is complex requiring not only the initiation of extensive cell death to remove the milk-producing epithelial cells but also the controlled influx of macrophages and other immune cell types to remove the dead cells, residual milk, and debris. These events are coupled with the breakdown of the extracellullar matrix, remodeling of blood vessels, and re-differentiation of adipocytes to regenerate the fad pad.

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دراسة مقارنة شكليائية نسجية للغدة اللبنية في الحوامل والمرضعات من الارنب المحلي (*Oryctolagus Cuniculus*)

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الخلاصة

الهدف من الدراسة :الوصف النسيجي للغدة الثديية في فترتين عمريتين (الغدة الثديية لارانب الحوامل والمرضعة).

طرق العمل: لتوضيح الفروقات التي يمكن أن تحدث في الغدة الثديية بين فترتين من حياة الأرانب المحلية (Oryctolagnus Cuniculus) لإجراء مثل هذا المشروع ، تم جمع 10 أرانب (5 لكل عمر) ولدراسة الفروق في الغدد الثديية حسب الصفات التشريحية والنسيجية للأرانب.

النتائج : كانت الغدد الثديية الظاهرة في الأرانب أربعة أو خمسة أزواج ، وتقع على السطح البطني للصدر والبطن والأربية. تحتوي الغدة الثديية للارانب على 8 أو 10 حلمات. احتوت النتيجة النسيجية لاناث مجموعة الحوامل على نفس المكون النسيجي ولكن كان هناك نسيج ضام الحويصلي داخل الفصيص من النسيج الضام بين الفصيصات ، والقناة الغدية ، والأنسجة الدهنية ، وبطانة الحويصلات مع الخلية المكعبة ، والقنوات المبطنة بطبقة مزدوجة من الخلية المكعبة حولها ألياف الكولاجين والفجوات الدهنية أظهرت نتائج هذه الدراسة أن فترة الحمل كانت الأكثر تطوراً وخاصة في الوحدات الغدية (الحويصلات) بسبب تأثير هرموني الإستروجين والبروجسترون في نمو القناة والحويصلات وتوقفها مباشرة بعد الولادة بعد سقوط المشيمة أيضا الكشف عن إفراز اللبأ. في مجموعة أرانب المرضعات ، كانت الفصيصات عبارة عن قنوات تجميع كبيرة مبطنة بطبقات متعددة من الخلايا العمودية والمكعبة وفصيص الغدة الثديية. أظهر المقطع المكبر من الحويصلات الغدية أنها مبطنة بخلايا إفرازية مكعبة منخفضة واحتوى تجويفها على منتج إفرازي وحويصلات غدية مبطنة بوحدات إفرازية مكعبة منخفضة واحتوى تجويفها على منتج إفرازي. تحتوي الحويصلات على تجويف كبير ومبطن بظهارة مكعبة ونواة مسطحة من الخلايا الظهارية العضلية ونواة مسطحة للخلايا الظهارية العضلية ، وتحتوي الفصيصات على الأنسجة الدهنية وكانت الفصيصات باحجام مختلفة والفصوص الملينة بالافرازو النسيج الضام الكثيف غير المنتظم والنسيج الضام بين الفصيص والأنسجة الدهنية ، والفصيصات باحجام مختلف. القناة بين الفصوص مملوءة بالافراز وحتى مجرى داخل الفصيص ،وقد لوحظ بعض الارانب في فترة الرضاعة كانت الصور النسيجية في معظم الحويصلات كانت كبيرة وبدون إفرازوهذا يدل على نهاية فترة الرضاعة او الانقلاب.

الاستنتاج: خلال عملية تكوين الثدي تمت دراسة النسيج الغدي للغدة الثديية ، قد تبين الغدة في بداية الحمل يزداد في التطور ويصل قيمته القصوى خلال 25-30 يوما من الحمل .

الكلمات المفتاحية: الأرانب ، الغدة الثديية ، الأرانب الحامل ، الرضاعة والانقلاب.