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Immunohistochemical and ultrastructural evidence for telocytes in the different physiological stages of the female rat mammary gland

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ABSTRACT

Introduction: Telocytes (TCs) are recently described to integrate a variety of different cells.

Aim of the work.

The aim was to investigate the presence of TCs in the rat mammary gland at its different physiological stages.

Material and methods: Twenty four adult female albino rats were classified into 4 groups: resting, mid-pregnancy, lactating, and involution groups. Inguinal mammary glands were processed for immunohistochemical and transmission electron microscopic (TEM) examination.

Results: TCs were immune-positive for c-kit and CD34 and showed significant differences in the different studied groups indicating variable roles at the different stages. TEM results characterized TCs by its shape and the long slender and moniliform telopodes linking the cells into stromal networks. The extracellular exosomes, homocellular synapsis and hetero-cellular synapsis were observed.

Conclusion: Our study provides evidence for the presence of TCs in all stages of the gland; not only in the resting stage as proved by other studies, but with immune-labeling differences suggesting different structural and physiological roles of TCs according to the stage requirements. These functions might via controlling the proliferation during pregnancy and lactation and the involution of the gland after weaning. Thus, more future functional studies of TCs will be important to help understanding the mechanism by which TCs contribute to tissue homeostasis concerning the role of the stromal/epithelial interactions in mammary gland biology and pathology including breast cancer which would be revolutionary for future therapeutic applications.

1. Introduction

Normal growth, function, and homeostasis of the epithelial cells in the mammary gland depend on the interactions between the numerous stromal cells within the gland. The stroma is composed of a mixture of cell types including the vasculature, adipocytes, resident immune cells, and fibroblasts [1].

The recently described interstitial cells called telocytes (TCs), formerly known as interstitial Cajal-like cells, have been found in almost all organs of the human body [2]. They are characterized by small and fusiform cellular body and typical elongated interconnected processes called telopodes [3]. Histologists considered it as interstitial cells that form a functional network, whereas it was considered as pacemaker cells by the physiologists [4]. TCs have been defined by their ultrastructure morphology [5] and specific immune-staining [6]. The immune-staining with CD34 [7] or c-kit [8] represents a useful markers for TCs. However, a unique marker that universally recognizes TCs has not been identified [3].

Ultrastructure morphology of TCs was characterized by small cell body with dark irregular heterochromatic nucleus that surrounded by a small amount of cytoplasm. The cytoplasm is rich in mitochondria, has small Golgi complex, rough and smooth endoplasmic reticulum and cytoskeletal elements. Their telopodes have dilated (podoms) and thin (podomers) portions [9] and establish intercellular contacts with various types of cells [10]. The dichotomous branching pattern of telopodes forms three-dimensional network through multi-points of contacts [11]. The TCs shapes depend on the number of telopodes: pyriform for one telopode, spindle for 2 telopodes, triangular for 3 telopodes, and stellate for > 3 telopodes [12].

Functionally, TCs play an important role in maintenance and regulation of the micro-environmental homoeostasis [13] due to their strategic position among target cells, blood capillaries, and nerve endings forming direct homo-cellular (between TCs) or hetero-cellular (between TCs and target cells) junctions. Also their biological functions

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might be performed in patterns of juxtacrine and paracrine effects by shedding extracellular micro-vesicles [14].These mechanisms might lead to fast adjustment in a variety of conditions [15]. Although several studies reported the existence of TCs in a wide variety of organs and tissues in the last few years [2], the biological functions of TCs remain unclear.

The mammary gland is a highly dynamic tissue in the body and shows high postnatal plasticity [16]. During puberty, mammary gland undergoes epithelial growth, branching, proliferation and invasion. While secretory differentiation and regression occur during pregnancy, lactation, and involution [17]. Unfortunately, those normally occurring processes occurs also in neoplasia of the gland. Fibrous tissue stroma communicates between mammary epithelia and their environment during the development. Disruption of the communication can induce cancer. Stromal factors essential for the development can promote or protect against breast cancer [18].

Virgin mammary gland is more susceptible to carcinogenesis, while early pregnancy protects against cancer development [19]. This might be due to the residual stem cell or progenitor population not removed by terminal differentiation occurs during pregnancy and lactation [16]. TCs and mammary stromal cells facilitated the formation of nest structure, promoted proliferation of breast cancer cells and inhibited apoptosis [20]. Therefore, the aim of this work was to investigate the possibility of the presence of TCs in the rat mammary gland at its different physiological stages e.g. resting, mid-pregnant, lactating and involuting stages in order to characterize them immuno-histochemically and morphologically. This may pave the future in concerning the role of the stromal/epithelial interactions in mammary gland biology and pathology.

2. Material and methods

2.1. Ethics

The experimental protocol was approved by the animal care committee of Faculty of Medicine-Minia University (Approval No.157: 2/2019) according to the international guidelines (Act 1986).

2.2. Animals and the experimental design

This study was conducted in Department of Histology and Cell Biology, Faculty of Medicine, Minia University. It was carried on 24 adult female albino Wistar rats (150–250 g and 8–10 weeks) obtained from the animal house of faculty of agriculture, Minia University. Animals were housed in clean plastic cages and fed a standard laboratory diet with free access to water and diet at room temperature in normal light/dark cycles. Rats were classified into 4 groups (6 rats each):

1. The resting group: Adult non-pregnant virgin rats which were bred separately from males (mammary gland appears as tubuloalveolar gland with prominent branched tubular ducts and fewer alveoli embedded in adipose tissue) [17].
2. The mid-pregnancy group: Pregnant rats were sacrificed on day 14 of gestation (the time the alveoli of the gland have increased in size and number of branching, less adipocytese and moderate lipid accumulation). Day 1 of gestation was identified by the appearance of spermatozoa in the vaginal smear [17,21].
3. The lactating group: Lactating rats were sacrificed on day 5 of lactation (as marked increase in cell number occurs during lactation with a peak during the first few days). The day of birth was considered day 0 of lactation [17].
4. The involution group: Weaning was occurred after 1 weak of lactation, then the post weaning involuting rats were sacrificed on days 7 after weaning (as the alveoli become disorganized and the amount of stroma surrounding alveoli rapidly increases) [17,22].

In each group, both sets of inguinal mammary glands were excised and divided for separate processing.

2.3. For immunohistochemical study

- Tissue samples were fixed in 10% neutral buffered formalin for 24 h, paraffin embedded and sectioned at 5 μm thickness.

The anti c-kit polyclonal antibody (CD117; Dako, Glostrup, Denmark) at 1:500 dilution used to characterize TCs as it is the most specific marker for TCs [23] and anti- CD34 antibody 1:150 (DAKO, Glostrup, Denmark) to investigate the TC ability to differentiate as progenitor cells [24] according to the manufacturer's guidelines. In brief, sections were deparaffinized, rehydrated, and incubated with trypsin then washed with PBS. The nonspecific binding was blocked in normal goat serum (1:50). Then it incubated for 30 min with the primary, washed with PBS, incubated with the secondary antibody (Vector laboratory 1:2000), and then avidin/biotin peroxidase complex (Vector, Burlingame, CA), which was detected using chromogenic 3,3′-diaminobenzidine (DAB) tetra hydrochloride substrate. Tissue sections were lastly counterstained with hematoxylin [25].

- Negative controls were done without adding the primary antibody. A positive control for c-kit was the tonsil and for CD34 was the capillary endothelium (figures not included).

2.4. For transmission electron microscopic (TEM) study

Smaller tissue pieces of 1 × 1 mm thickness were fixed in 4% cold glutaraldehyde for 48 h and washed with phosphate buffer (BPS). The fixed tissues were postfixed with buffered 1% osmium tetroxide and embedded into an epoxy resin. Semithin sections of 1 μm thick were cut and stained with 1% toluidine blue. Ultrathin sections were cut and double-stained with uranyl acetate and lead citrate [26].

2.5. Image capture

- Immune-stained and semithin sections were examined using Olympus microscope (Olympus, Tokyo, Japan) and images were digitally captured using a high-resolution color digital camera (Olympus, Tokyo, Japan) mounted to the microscope and installed to a computer.
- Ultrathin sections were examined with transmission electron microscope (TEM) (JEOL, Tokyo, Japan) and photographed at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Egypt.

2.6. Morphometric study

Immune-stained sections were examined in all the studied groups. The number of c-kit and CD34 immune-positive TCs × 40 magnifications was assessed manually (to avoid counting other immune-positive cells as endothelial cells or mast cells) in 10 non-overlapping fields per section for each rat (n = 6).

2.7. Statistical analysis

The numerical data obtained were statistically analyzed using SPSS (IBM, Version 20). Values were expressed as mean ± standard deviation (SD). The significance of differences was assessed by Student's *t*-test and p-value ≤ 0.05 was considered statistically significant.

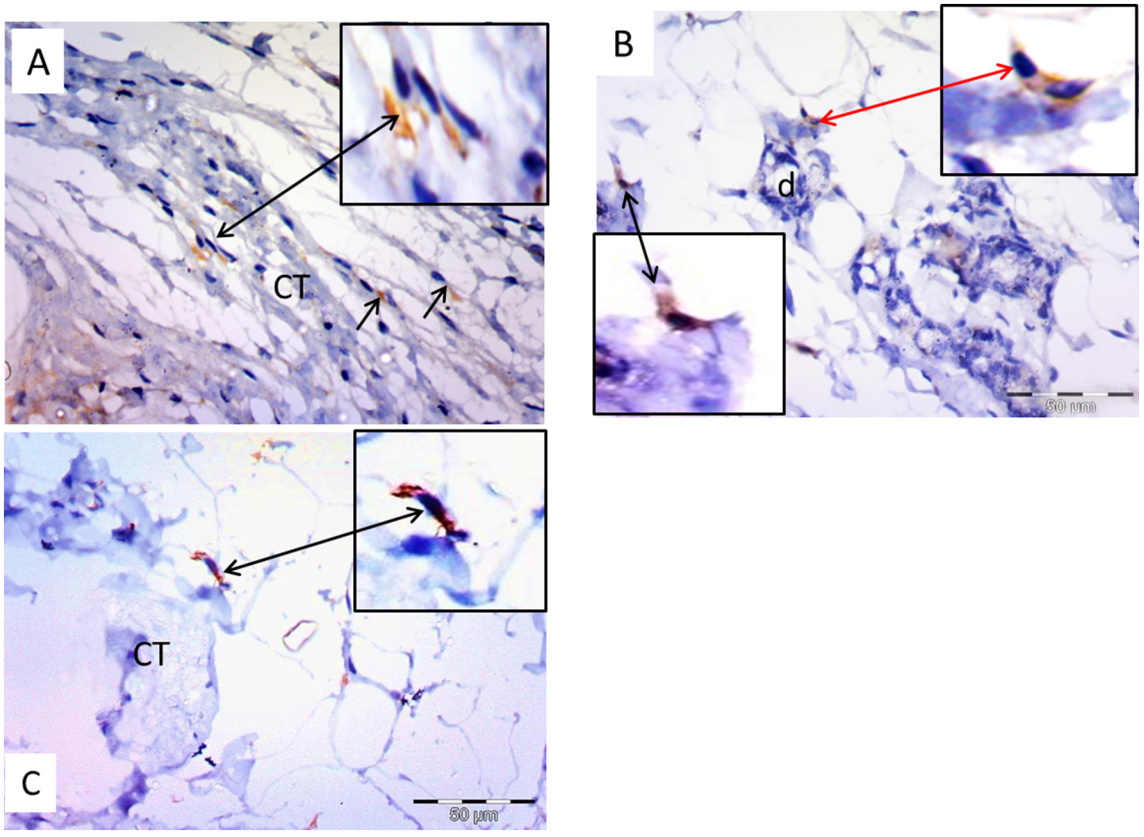

Fig. 1. Photomicrographs of rat mammary gland from the resting group showing: A) numerous c-kit immune-positive TCs (arrows) of variable shapes with oval nuclei and extended immune-positive telopodes within the CT (CT). B) c-kit immune-positive spindle shaped single TCs (black arrow) or in close to each other TCs (red arrow) in the CT surrounding ducts (d). C) CD34 immune-positive spindle shaped TC (arrows) in the CT (CT). Notice the oval nuclei and bipolar cytoplasmic processes. Insets are higher magnification of the immune-positive cells.
Immunohistochemistry, counterstained with H: X400, Insets X 1000. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Immunohistochemical results

The immunohistochemical staining of sections of the mammary gland for anti c-Kit and anti CD34 antibodies showed that:

In the resting group, many c-Kit immune-positive TCs were observed in the interlobular connective tissue (CT) and surrounding ducts. TCs were either spindle shaped with oval nuclei and bipolar cytoplasmic processes (telopodes) or pyriform with triangular nuclei and a single extended telopode. They were located singly or in groups close to each other. However, fewer CD34 immune-positive cells with telocyte-like morphology were noticed in the CT of this group (Fig. 1).

In the mid-pregnancy group, the c-kit immune-positive cells appeared with multiple ramified processes at the interface between CT surrounding ducts, fat cells and in the CT surrounding the small developing alveoli. Interestingly, TCs in areas of well-developed alveoli were small with short processes. Sections immune-stained for CD34 antibody revealed increased immune-positive TCs (Fig. 2).

The lactating group had fewer c-Kit and CD34 immune-positive cells with telocyte-like morphology. The positive cells had oval or pyramidal nuclei and long telopodes which were usually interconnected (Fig. 3).

In the involution group, numerous c-Kit and few scattered CD34 immune-positive TCs were observed in the CT surrounding the involuting alveoli, ducts and among interlobular CT. Most of them were spindle with oval nuclei and bipolar processes. Others were pyriform in shape with single process (Fig. 4).

3.2. Morphometrical results

The c-kit immune-stained sections showed no significant differences in the mean number of positive cells of the mid-pregnant ($p = 0.501$) and the involution ($p = 0.091$) groups if compared to the resting group. While the lactating group showed a significant decrease if compared to the other 3 groups (all $p = 0.000$).

Regarding the CD34 immune-positive cells; there was a significant increase in the mean number of the positive cells of the mid-pregnant group if compared to the other 3 groups (all $p = 0.000$). While the lactating and the involution groups (all $p = 0.000$) had a significant decrease if compared to the mid-pregnant and the resting groups (all $p = 0.000$). Moreover, comparing the involution group to the lactating group showed a significant increase ($p = 0.021$) in the mean number of the positive cells (Table 1, and Fig. 5).

3.3. Semithin results

Semithin sections of the mammary glands at the different studied stages showed the characteristic variable shapes and also variable distribution of the TCs. TCs of the resting group were in the CT surrounding ducts, in the mid-pregnancy stage were observed near the developing alveoli, in the lactating group were located in the CT among the alveoli, and in the involution stage were observed in-between the involuted alveoli. Most of TCs were spindle in shape with their long telopodes located within the CT (Fig. 6).

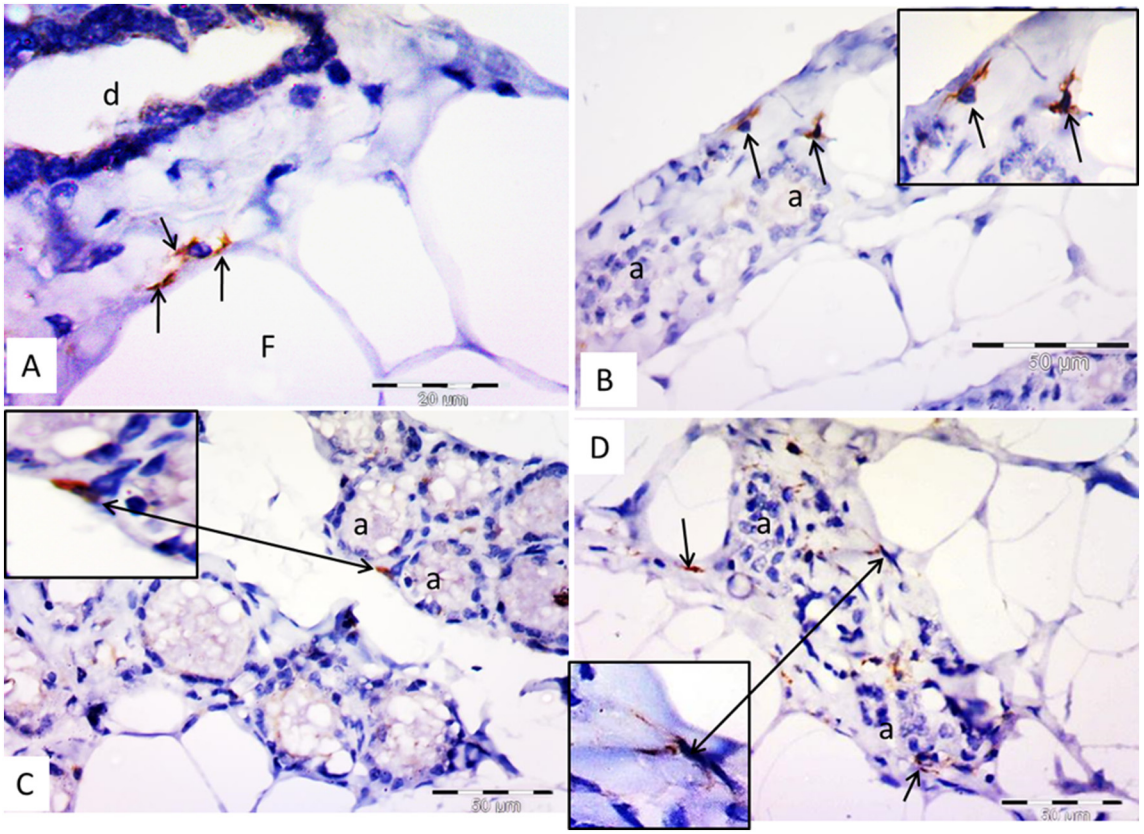

Fig. 2. Photomicrographs of rat mammary gland from the mid-pregnancy group showing: A) c-kit immune-positive TCs with multiple processes (arrows) at the interface between a duct (d) and fat cells (F). B) c-kit immune-positive TCs with multiple processes in the CT surrounding small developing alveoli (a). C) c-kit small immune-positive TC with short process (arrow) closes to large alveoli (a). D) CD34 immune-positive TCs (arrows) in the CT surrounding small developing alveoli (a). Insets are higher magnification of the immune-positive cells.
Immunohistochemistry, counterstained with H: A& insets X 1000; B,C& D X400.

3.4. The TEM results

Electron photomicrographs (digitally colored) of the resting group showed pyriform TCs (0–2 cells/Field of view) with single moniliform telopode near ducts (50%). Some telopodes were noticed running between bundles of collagen fibers between lobules (50%). Other TCs were spindle shaped with 2 telopodes. The telocyte body had thin cytoplasm with few organelles and large heterochromatic nuclei. The telopodes were cylindrical and long with areas of dilatations (podoms). Multivesicular bodies, mitochondria, and few cisternae of the endoplasmic reticulum were noticed within the cytoplasm. The characteristic dichotomous pattern of telopode branching was obviously seen (Fig. 7).

The mid-pregnancy group showed triangular TCs (0–2 cells/Field of view) with large heterochromatic nuclei and thin cytoplasm with few organelles in close vicinity to ducts and alveoli (75%). Exosomes were observed in the interstitium close to the telopodes (Fig. 8).

The lactating group showed stellate shaped TCs (0–3 cells/Field of view) with irregular heterochromatic nuclei and thin cytoplasm with few organelles in close vicinity to ducts and blood capillaries (80%). They formed multiple and different sites of close contacts forming homo-cellular (between 2 TCs) and hetero-cellular junctions (between TC and duct cells or TCs and endothelium of blood capillaries). Their Telopodes were thin, long, and beaded with alternating podoms and podomers. Exosomes were observed either attached to it or sheded in their vicinity (Fig. 9).

The involution group showed pyriform TCs (0–3 cells/Field of view), each had single moniliform slender telopode which were seen running between bundles of collagen fibers of the interalobular CT (50%). Their cytoplasm had few organelles and large heterochromatic nuclei. The telopodes had mitochondria and multivesicular bodies. Other sections showed numerous TCs with their extended long slender telopodes communicate each other and exosomes were observed near them in the interlobular CT (50%) (Fig. 10).

4. Discussion

Mammary gland is a unique gland that differs from other organs in that it continues to undergo postnatal morphogenesis with paramount changes in tissue structure and cell population dynamics [27] occurring during puberty, pregnancy, lactation and involution periods [28]. More differentiated mammary gland during lactation exhibits high metabolic and synthetic activity, whereas during involution apoptotic cell death accounts for the return of the gland to the resting state [29]. Many factors modify the developmental profile of the gland which might modify breast cancer risk [16]. Thus the discovery of new players in the regeneration and reparation of this organ would be revolutionary for future therapeutic applications. This makes the mammary gland an interesting target to study its cells. One of the cells proved to be present in the resting mammary gland was the TCs [20]. To our knowledge, no study concerned TCs in the different physiological stages (resting, pregnant, lactating and involution stages) of mammary gland. Therefore the aim of this study was to localize TCs immunohistochemically and morphologically in the stroma of the gland at its different physiological stages.

The profile of immunohistochemical staining of TCs may be different between organs moreover, it may be different within the same location [14]. C-kit is a type III receptor tyrosine kinase operating in

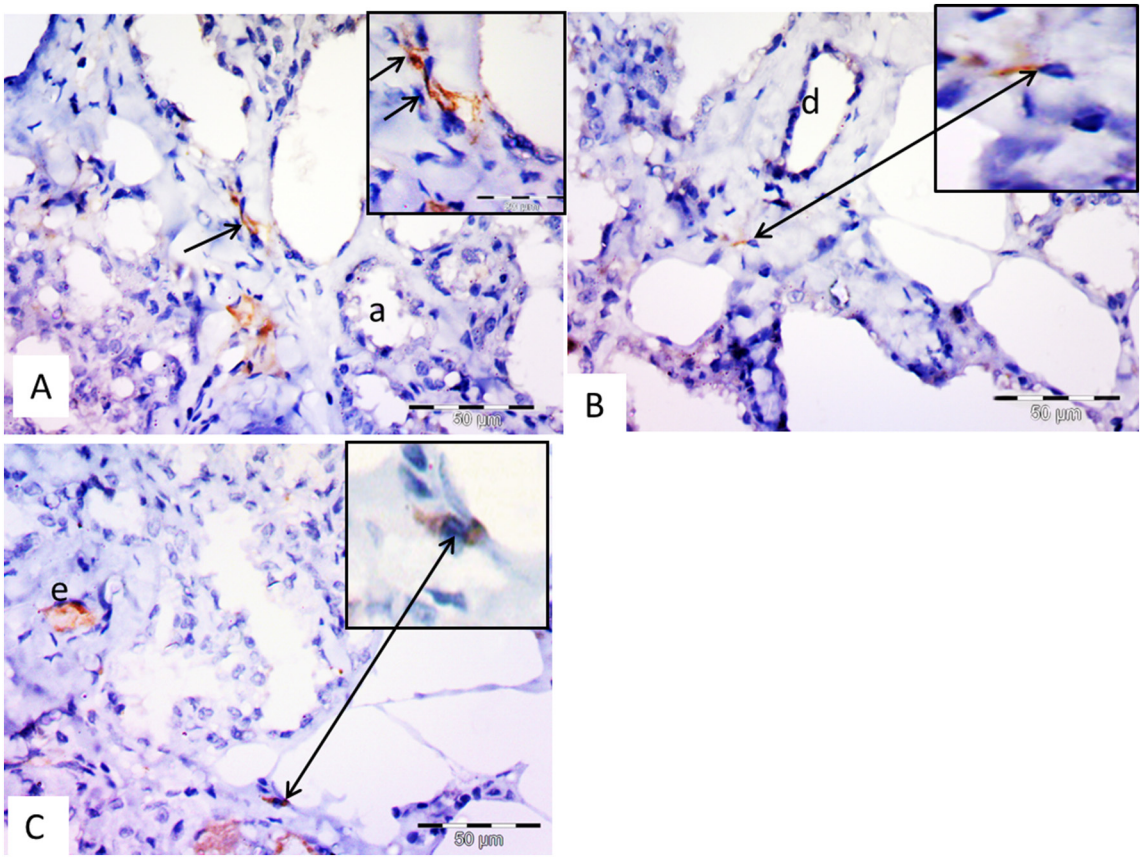

Fig. 3. Photomicrographs of rat mammary gland from the lactating group showing: A) c-kit immune-positive TCs with interconnected telopodes (arrows) in the CT between the alveoli (a). B) c-kit immune-positive pyriform TC in the CT surrounding a duct (d). The positive cells have pyramidal nucleus and a single long telopode (arrow). C) A spindle shaped CD34 immune-positive TC (arrow). Notice positive expression in the vascular endothelium (e). Insets are higher magnification of the immune-positive cells.
Immunohistochemistry, counterstained with H: X400; insets X1000.

cell signal transduction in several cell types. Normally, it is activated (phosphorylated) by binding of its ligand; the stem cell factor. This leads to a phosphorylation cascade ultimately activating various transcription factors in diverse cell types. This activation regulates cell differentiation, proliferation, apoptosis, chemotaxis and cell adhesion [30].

In this study, c-kit positive TCs were observed in the interlobular and intralobular CT (between the lobules and surrounding ducts & alveoli, respectively) and acquired different shapes either spindle, pyriform or stellate shapes. This was in line with the study of Popescu and his team [9] who identified and localize c-kit positive TCs among the stromal cells in the resting human mammary gland. TCs provide mechanical support that directs cell migration during development, repair and renewal of an organ [31]. This might explain the significant differences observed in the studied groups according to the variable functions required for the physiological needs of the stage that could performed by TCs. The observation of c-kit positive cells in close vicinity to ducts and developing alveoli in the mammary gland of the pregnant rats might support the close relationships between sub-epithelial TCs network and adjacent epithelium mentioned by Briton-Jones and his team [32]. This might be mediated by c-kit as binding of c-kit receptor to stem cell factor induce downstream signal transduction pathway which subsequently regulates gene expression and cell growth, proliferation and differentiation [33].

CD34 was predominantly regarded as a marker of hematopoietic stem cells and hematopoietic progenitor cells. However, CD34 is now established as a marker of several other non-hematopoietic cell types and a common marker for diverse progenitors [24]. Although Petre and his colleagues [34] found TCs negative for c-kit, they were agreed with the results of this study in the positive immune-reactivity of TCs to CD34. They added that CD34-positive TCs built stromal networks that ensheathed microvessels and excretory units in the resting mammary gland. In this study, there were significant differences comparing the mid-pregnant group to the resting, lactating group and the involution group. At this stage, rapid and continuous increase in the mammary gland epithelium with growth of both lobules and ducts occurred [17]. Researchers [34] suggested that CD34-positive TCs of the mammary gland stroma could be actors in the mammary stem niche and different antigen expression might relate to different stages of differentiation. It was also suggested that TCs could be transit amplifying cells deriving from neighbor stem cells that might justify their positive CD34 and c-kit phenotypes. This could interpret the more numerous CD34-positive TCs which were notably recognized during the mid-pregnant stage. Many theories indicate that TCs could behave as progenitor cells, thus transit amplifying cells [35] which could explain the significant decrease of CD34 immune-positive cells in the lactating and involution groups as a matter of consumption without further need in these stages. However, several studies proved that TCs are functionally distinct from mesenchymal stem cells with regard to their gene expression profile [36], and the chromosomal analysis also revealed that specific genes in TCs are different from those of mesenchymal stem cells [37].

Taken together the previous suggestions and the immunohistochemical findings of this study, it could be assumed that TCs might play a critical role controlling the structural and functional remodeling from one stage to the other either by the proliferation during the preparation of the gland for lactation or controlling apoptosis

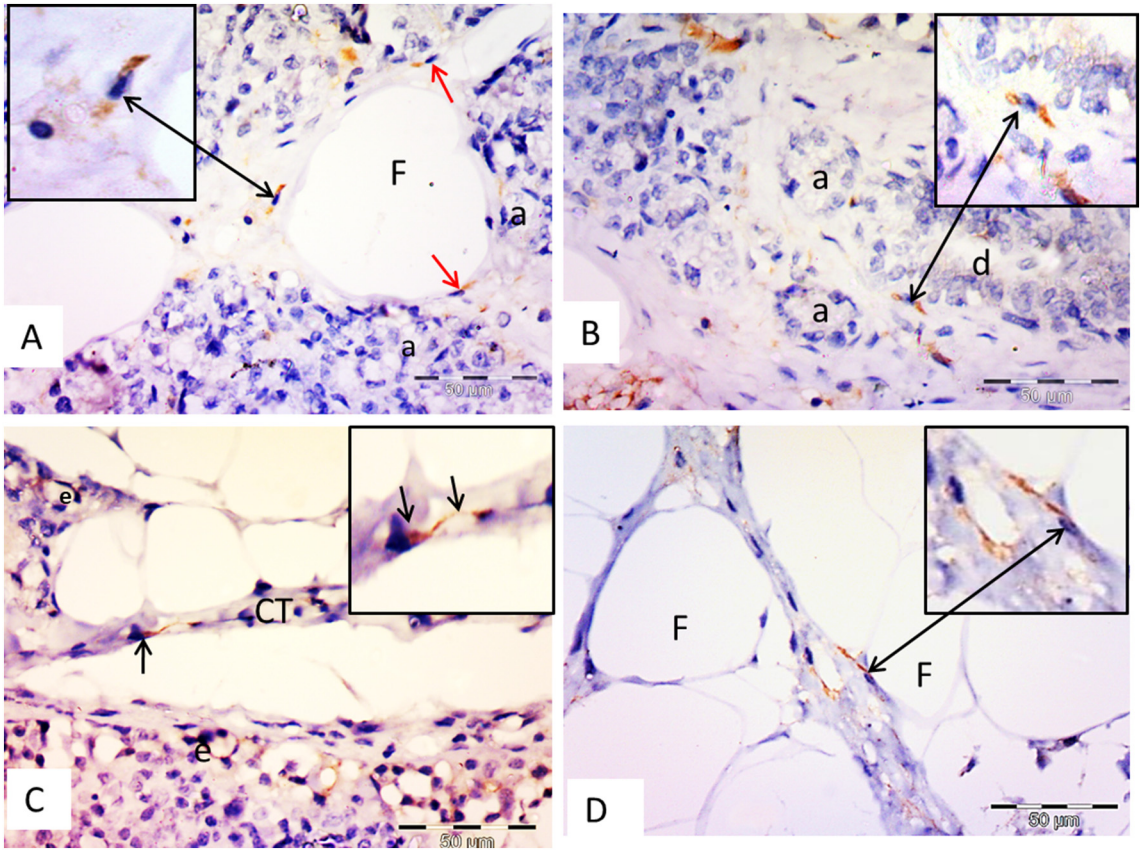

Fig. 4. Photomicrographs of rat mammary gland from the involution group showing: A) c-kit immune-positive spindle TCs with oval nuclei and bipolar cytoplasmic processes (black arrow), others with single processes (red arrows) in the CT surrounding the involuting alveoli (a). B) c-kit immune-positive TC (arrow) with bipolar processes in the CT surrounding a duct (d). C) c-kit immune-positive pyriform TC with single long process (arrows) in the CT between the lobules. Notice positive expression in the vascular endothelium (e). D) CD34 immune-positive TCs (arrows) within the adipose CT (F). Insets are higher magnification of the immune-positive cells.
Immunohistochemistry, counterstained with H: X400; insets X1000. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Mean number of immune-positive cells in the studied groups (n = 6).

	Mean ± SD	p-value
c-kit immune-positive cells		
1. The resting group	33.17 ± 1.94	
2. The mid-pregnancy group	32.5 ± 0.54	0.501^{r}
3. The lactating group	17 ± 1.41	0.000^{r}*
		0.000^{p}*
4. The involution group	31.67 ± 0.82	0.091^{r}
		0.141^{p}
		0.000^{l}*
CD34 immune-positive cells		
1. The resting group	16.16 ± 0.75	
2. The mid-pregnancy group	27.5 ± 1.87	0.000^{r}*
3. The lactating group	8.16 ± 1.17	0.000^{r}*
		0.000^{p}*
4. The involution group	10.33 ± 1.03	0.000^{r}*
		0.000^{p}*
		0.021^{l}*

[r] vs the resting group
[p] vs mid-pregnancy group
[l] vs lactating group.
* $p \leq 0.05$ is significant.

during the involution of the gland after weaning. Owing that to the specific roles of TCs in cell signaling, tissue homeostasis, remodeling and angiogenesis which proved by Zheng et al., [36] and Varga et al., [38]. Thus TCs were seen as future targets with implications for regenerative medicine [39].

In the current study, by using the TEM, TCs were found between the tubulo-alveolar structures in the different stages of the study. It was identified with its TEM characteristics described by Popescu and his colleagues [9]. In all the studied stages, TCs appeared with small cell bodies, thin cytoplasm, large heterochromatic nuclei, and their unique cytoplasmic moniliform processes that called telopodes. In this study, numerous shapes were observed in the different studied stages. Presumably, the spatial appearance of TCs would be depending on their telopode number [40].The telocyte's hallmarks; telopodes, appeared thin, long, beaded and connected forming networks. Telopodes had alternating dilated segments; podoms, and thinner segments; podomers [14,40]. Podoms are small functional units capable of synthesizing protein and interacting with target tissue cells epigenetically through bioelectric communication [41]. In the results of this study, only 1 to 3 telopodes were observed on a single TEM section. Its site, the angle of section, and their convolutions did not allow their full three-dimensional observation in a two-dimensional section.

TCs are considered as a critical stromal structure of the interstitial morpho-functional unit and this necessarily requires range of forms of information transfer mechanisms [42]. TCs might be involved in intercellular signaling, taking into account the 3D network of telopodes and their strategic position in between target cells [14]. Here TCs were seen in close association to other TCs, fat cells, duct cells and endothelial cell. Hence, it might play a pivotal role in integrating the stroma as a functional assembly. Other mechanisms that could be considered included a paracrine and/or a juxtacrine secretion of small

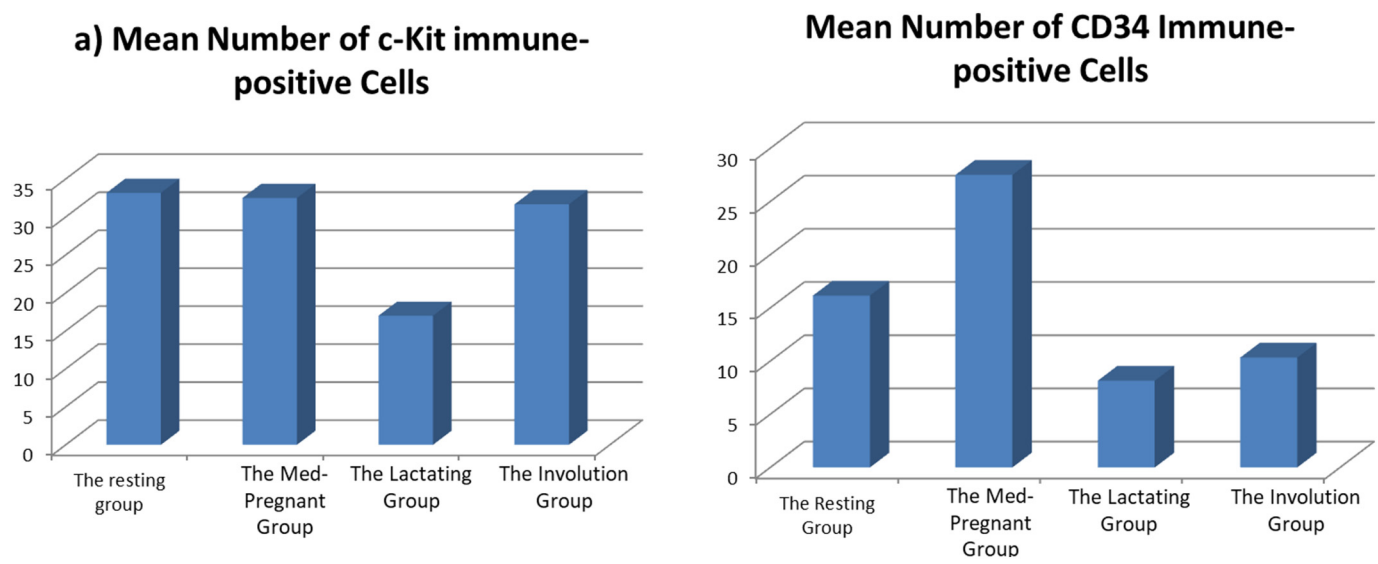

Fig. 5. Mean number of immune-positive cells in the studied group (n = 6).

signal molecules and shedding of microvesicles (exosomes) [14,43]. These exosomes were seen in the environment surrounding TCs in the different studied stages. It could be suggested that exosomes were specific containers filled with proteins, lipids, microRNA and/or other materials that are transported to other neighboring stromal cells where they alter their function and physiology. Some studies assumed that exosomes are derived from both the cell body and the telopodes of TCs [44]. In this study, the microvesicles observed in the vicinity of TCs

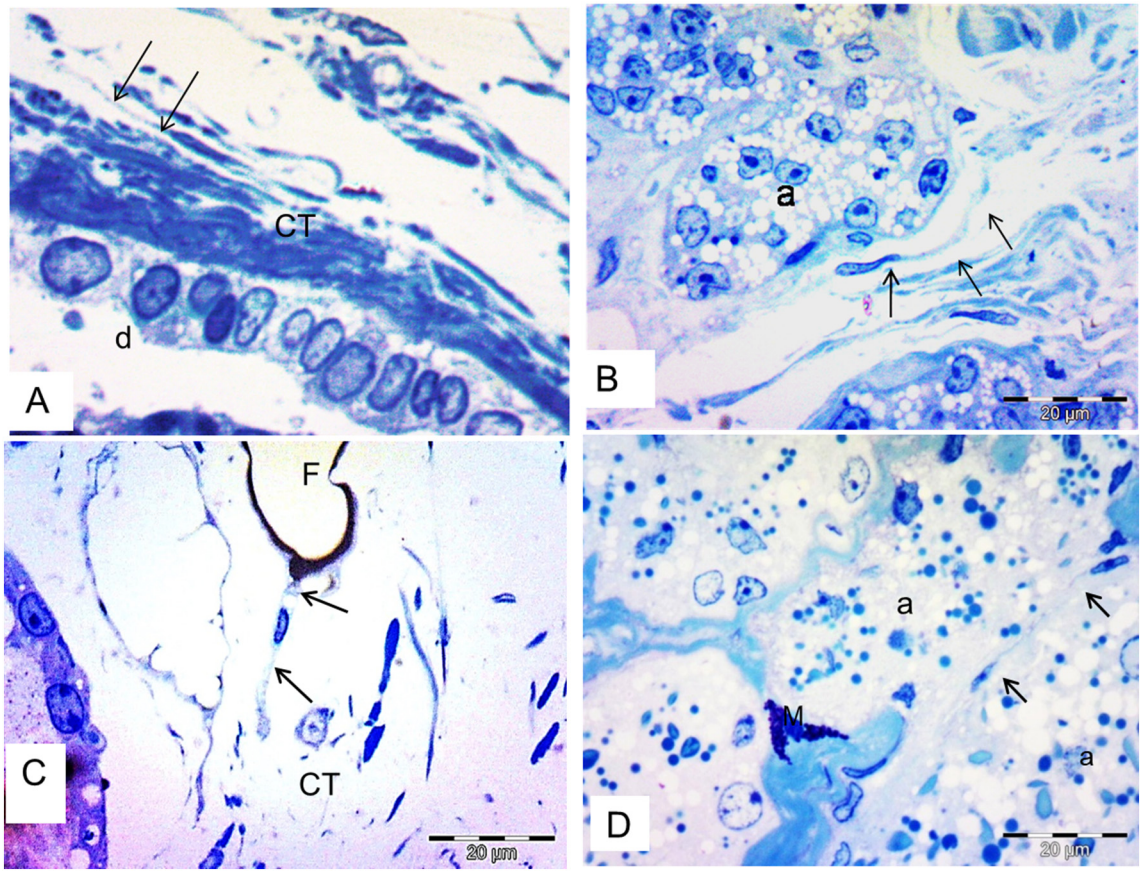

Fig. 6. Semithin sections of rat mammary gland showing:
A) A telocyte from the resting group with long telopode (arrows) within the CT surrounding a duct (d).
B) A telocyte from the mid-pregnancy group with long telopode (arrows) very close to secretory alveoli (a).
C) A spindle shaped telocyte from the lactating group with two telopodes (arrows) close to fat cell (F).
D) TCs from the involution group of spindle shaped bodies and thin telopodes (arrows) surrounding the involuted alveoli (a). Notice a mast cell with characteristic granules (M).
Toluidine blue X1000. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

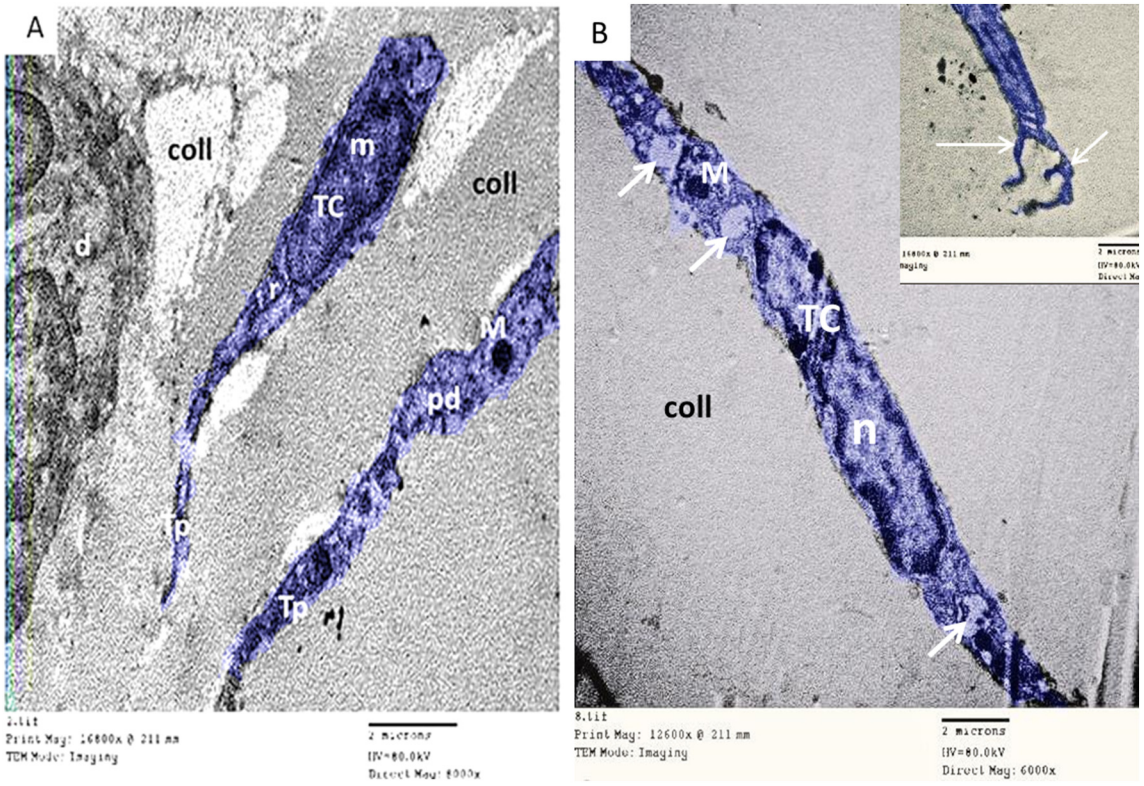

Fig. 7. Digitally colored electron micrographs of rat mammary gland from the resting group showing:
A) A pyriform telocyte (TC) with single moniliform telopode (TP) and a telopode (TP) of other cell running between bundles of collagen fibers (coll) next to a duct (d).
B) A spindle shaped telocyte (TC) with two telopodes (TP).
The telocyte body (TC) has thin cytoplasm with few organelles and large heterochromatic nucleus (n). The cytoplasmic processes are slender long extensions with areas of dilatations; podomes (pd), multivesicular bodies (arrows), mitochondria (M), and few cisternae of the endoplasmic reticulum (r).
Notice the dichotomous pattern of telopode branching (arrows in inset).
A, inset X8000; B X6000.

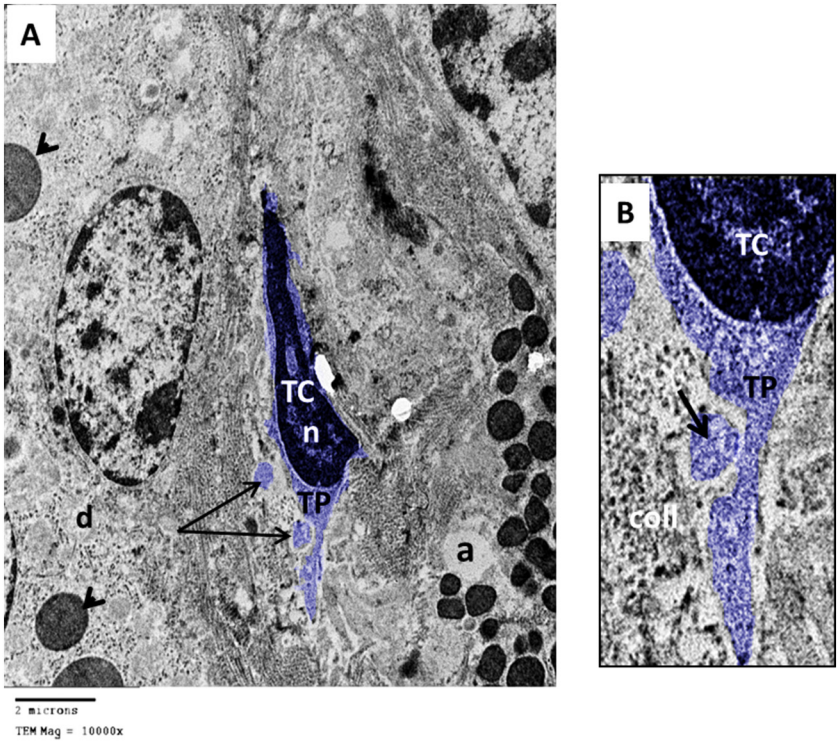

Fig. 8. Digitally colored electron micrographs of mammary gland from the mid-pregnancy group showing:
A) A triangular telocyte (TC) with large heterochromatic nucleus (n), thin cytoplasm with few organelles in close vicinity to a duct (d) and acinus (a). Notice telopodes (Tp) with exosomes (arrows) in the interstitial space, and the characteristic lipid droplet of the duct cell (arrowheads).
B) A higher magnification showing the telopode (Tp) and the exosomes (arrow) surrounded by collagen (coll).
AX10000, BX25000.

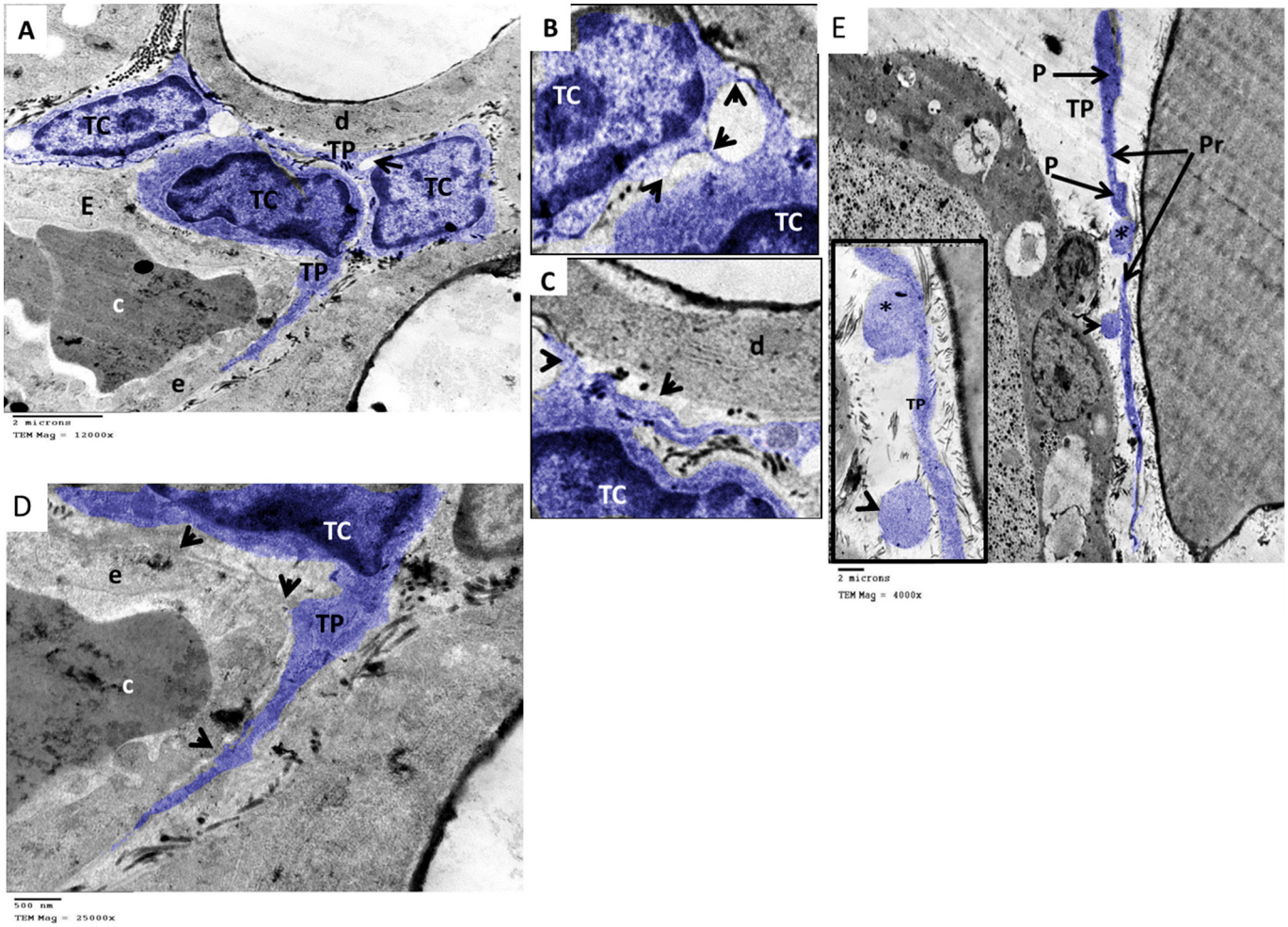

Fig. 9. Digitally colored electron micrographs of mammary gland from the lactating group showing:
A) Telocytes (TC) with stellate shape bodies occupied by irregular heterochromatic nucleus (n) and thin cytoplasm with few organelles close to a duct (d) and blood capillary (c).
(B-D) Higher magnifications showing multiple and different sites of close contacts (arrowheads) between: B) two telocytes (TC) C) telocyte (TC) and duct cells (d), and D) TC and endothelial cell (e).
E) A telopode (TP): thin long beaded with alternating podomes (p) and podomers (pr). Notice the attached exosomes (*) to the telopode and the sheded (arrowhead) in the close vicinity of the telopode (inset).
AX12000; B,C,DX25000, EX4000 & inset X15000.

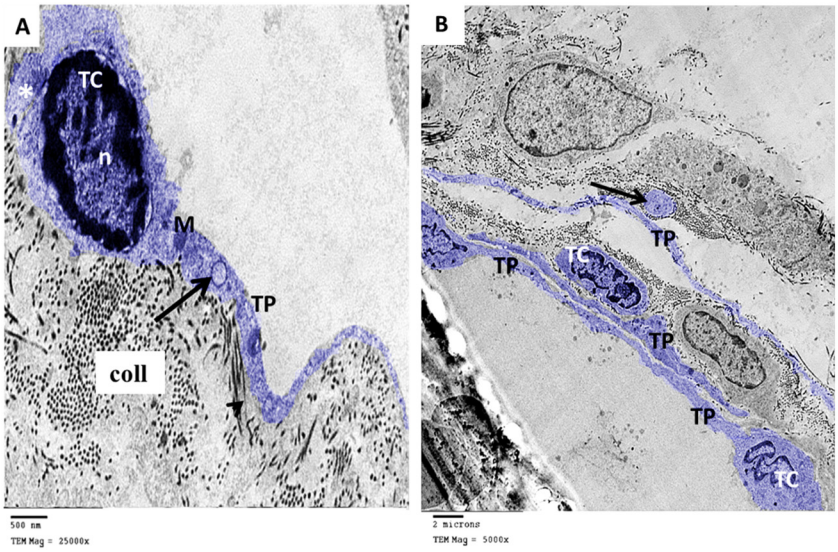

Fig. 10. Digitally colored electron micrographs of rat mammary gland from the involution group showing:
A) A pyriform telocyte (TC) with single moniliform slender telopode (TP) running between bundles of collagen fibers (coll). Notice the cytoplasm (*) with few organelles and the large heterochromatic nucleus (n). The telopode has mitochondria (M) and a multivesicular body (arrow).
B) Numerous telocytes (TC) with their extended long slender telopodes (Tp) communicate each other. Notice the exosome (arrow) near a telopode (TP).
AX25000; BX5000.

could be one of the mechanisms through which TCs meet target-tissue requirements. This might lead to rapid phenotype adjustment in the different morphological stages of the mammary gland. TCs release exosomes in vivo and in vitro suggesting that TCs regulate the activity of other cells by vesicular paracrine signals. Moreover, there is a continuous, post-transcriptional regulatory signal between TCs and stem cell as TCs deliver microRNA to stem cells through exosomes and stem cells deliver microRNA loaded extracellular vesicles to TCs [45]. This could be supported by other research where endothelial cells that cultured with telocyte's exosomes exhibited increased proliferation and formation of capillary-like structures [46]. Also exosomes were proved to have roles in stem cell maintenance, tissue repair, immune response and vascular hemostasis [42].

Numerous studies described the ability of TCs to direct interact with themselves by homo-cellular junctions and with other important surrounding structures such as blood vessels, nerve endings, smooth muscles, glandular elements, and the covering epithelia by hetero-cellular junctions [44,47]. This was clear in the results of this study where TEM results provided evidence that TCs frequently established close contacts (synapses) with several types of cells in the rat mammary gland during its several physiological stages. Both homo-cellular and hetero-cellular synapses were observed which was in agreement with the results of Faussone-Pellegrini & Gherghiceanu [47] who mentioned that TCs build a scaffold important for intercellular communication allowing the exchange of information and spreading of signals. While the hetero-cellular synapsis between TCs and various cell types give origin to mixed networks. TCs, by these types of contacts, serve as a bridge that directly links the adjacent cells through membrane-to-membrane contact [20]. Therefore, it could be suggested that it might have functional modulatory role controlling the conversion into the different stages of the mammary glands. These synapses occurred not only in the human breast [28] but also in the interstitial tissue of various organs [48]. Although, TCs are cells that capable of acting as integrators of many intercellular functions there is a long way ahead to elucidate their functional capabilities [38,39,43].

In conclusion, our study provides evidence for the presence of TCs in all stages of the gland; not only in the resting stage as proved by other studies, but with immune-labeling differences suggesting different structural and physiological roles of TCs according to the stage requirements. These functions might via controlling the proliferation during pregnancy and lactation and the involution of the gland after weaning. Thus, more future functional studies of TCs will be important to help understanding the mechanism by which TCs contribute to tissue homeostasis concerning the role of the stromal/epithelial interactions in mammary gland biology and pathology including breast cancer which would be revolutionary for future therapeutic applications.

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Declaration of Competing Interest

There is no conflict of interest to declare.

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