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Original Article

Ultrastructural and immunophenotype distribution of Telocytes in female genital tract of adult albino rats

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ABSTRACT

Background: Few information was given about Telocyte (TC) which was recently discovered in the last few years.
Aim of the work: This study was designed to give full histological, immunohistochemichal and ultrastructural preview on TCs distribution in the female genital tract.
Material and method: After vaginal smear examination, thirty rats in diestrus phase were subjected for this study. Uterine, fallopian tube and vaginal specimens were taken and processed for both light and electron microscopic examination.
Results: TCs showed weak positive ER-α and marked immunoexpression of PR-A and CD34 in the endometrial stroma and around the glands, in stratum vascular and outer longitudinal smooth muscle layers of the uterus, serosa of the fallopian tube and in the lamina propria in addition to the connective tissue under vaginal epithelium and around and within the bundles of the vagina. Ultrastructurally, the uterus, fallopian tube and vagina showed triangular or stellate- shaped TCs that contained elongated nucleus occupying the whole cell body with thin band of marginal heterochromatin and prominent nucleolus and had one or more telopodes (TPs) with dichotomous pattern of branching. TPs had thin podomere and thick podom. Podom contains many mitochondria, rough endoplasmic reticulum (RER), many caveolae and pinchedout vesicles. TCs had either homocellular junction between them or heterocellular junction with adjacent smooth muscle cells. TCs were surrounded by collagen fibers.
Conclusion:TCs are new type of cells with special ultrastructure and immunophenotype characters that are detected in the female genital tract ,therefore further studies on their distribution in the female genital tract during different stages of estrus cycle, and trying to correlate between their distribution and their role in controlling infertility or myometrial contraction during pregnancy should be considered.

Key Words: Fallopian tube; Immunohistochemistry; Telocytes; Ultrastructure; Uterus; Vagina.

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INTRODUCTION

A new type of cell which was known as "Intersitial Cajal-Like Cells (ICLC) was discovered during the last few years. They were taken this name due to their apparent similarity to the gastrointestinal interstitial cells of Cajal (ICC). However, it became evident that the ultrastructure of ICLC was completely different from that of ICC, as they have different ultrastructure and immunophenotype, and therefore should be functionally distinct (Popescu and Faussone-Pellegrini, 2010). hence, these cells were termed telocytes (TCs) while Telopodes (Tp) were applied to their extremely long and thin prolongations(*Gherghiceanu and Popescu, 2005a,b; Suciu et al., 2010a,b*).

It was mentioned that TCs were stromal; interstitial, cells of mesenchymal origin and they were thin, long moniliform and occasionally showed convoluted prolongations, that were different from other stromal cell types, such as fibrocytes, fibroblasts and fibroblast-like (*Popescu and Faussone-Pellegrini, 2010*). Tps appeared as cell prolongations which emerged directly from the cell body, and not a thick emergence of the cell body that became gradually thin as happens in antigen presenting cells, fibroblasts or myofibroblasts and neurons. Tp consists of thin segments called podomers and dilations termed podoms (*Bani et al., 2010; Carmona et al., 2011*).

The differences between TC and fibroblasts were distinct since they have different phenotypes and ultrastractural appearance. Therefore, their functions were also different: fibroblasts form collagen, while TC promote intercellular signaling either by direct contact (junctions), or remotely through extracellular vesicles. In other meaning, fibroblasts are more structurally oriented, responsible for collagen and extracellular matrix synthesis and fibrosis, whereas TCs are more functionally oriented, being involved in transcellular communication (*Ciontea et al., 2005; Popescu, 2011a*).

It was reported that TCs were found within the interstitium of many organs including the heart (*Kostin, 2010; Suciu et al., 2010a*), placenta (*Suciu et al., 2010b*), lung (*Zheng et al., 2011; Popescu et al. 2011c*), urinary tract (*Gevaer, 2012*), skeletal muscle (*Popescu et al., 2011d*), parotid gland (*Nicolescu et al., 2012*), pancreas (*Nicolescu and Popescu, 2012*). The exact role of TCs in these organs was suggested to be mechanical support (*Hinescu et al., 2008*), immune surveillance (*Carmona et al., 2011*), regulation of stem cell niche (*Gevaert, 2012*), and tissue regeneration (*Popescu et al., 2011a,b*) and intercellular signaling (*Gherghiceanu and Popescu, 2012*) .

Several markers have been identified on TC either by immunohistochemistry (IHC) or by confocal imaging. TC exhibited many markers which include mainly CD34, CD117/c-Kit, however electron microscope remains the method of choice to identify TC. The double positive immunostaining with CD34/c-Kit (mainly for cell body) or CD34/ vimentin (mainly for Tp) also represents a useful marker for TC (*Suciu et al., 2010b; Suciu et al., 2012*).

TCs were discovered in human and rat myometrium (*Ciontea et al., 2005a; Popescu et al., 2006; Cretoiu et al., 2011*) and established as cellular components of the hormonally responsive uterine tissue (*Popescu et al., 2007; Cretoiu et al., 2010; Hatta et al., 2012*) and in fallopian tube (*Popescu et al. ,2007*). Experimental studies suggested involvement of TCs in spontaneous contraction of the uterus (*Allix et al., 2008; Cretoiu et al., 2011*). This is possibly occured under the hormonal effect, as uterine TCs have been proved to exhibite estrogen and progesterone receptors (*Cretoiu et al., 2006*). Therefore; the present work aims to throw more light on the ultrastructure and immunophenotype distribution of telocytes as new cells, and try to correlate between their location and their expected role in female genital tract of adult albino rats.

MATERIAL AND METHODS

Animals and tissue sampling

Forty adult female albino rats were used for the present study. Rats were (4 months old and 200 -220 gm). The animals were maintained under conventional relative humidity, 12-h light/12-h dark, with free access to chow

and water. The rats were supplied from the animal house, Faculty of Medicine, Mansoura University. All rats were examined for the stages of estrous cycle which were determined by examination of vaginal smears. Thirty rats were in diestrous cycle and these rats were prepared for histological examination of their female genital tract. After anesthesia by an intraperitoneal injection with sodium thiopental (40 mg/kg of body weight) (Sigma Chemicals Co., St Louis, Missouri, USA), the uterus, fallopian tube and vagina were removed. Part of these organs were fixed by immersion in Bouin's solution for 24 hours followed by dehydration in ethanol gradient, clearance in xylene, embedding in paraffin and cutting into 5 µm sections. Histological sections were stained with H&E and immunohistochemical stains for localization of estrogen (ER-α), progesterone (PR -A) and CD34 expression by telocytes. Another small parts of these removed organs were fixed in glutaraldehyde and prepared for electron microscopic examination.

The experiment was executed in conformity with National Institutes of Health (NIH) guidelines for the maintenance and use of science lab animals; NIH Publication 1986 (86/609/EEC) and in accordance with local laws and ordinances.

Immunohistochemical technique

Strept- avidin-biotin-peroxidase was used to visualize ER-α, PR- A and CD34 (*Mote et al., 2001; Sahlin et al. ,2006; Zhenga et al., 2011; Blesson et al., 2012*). A monoclonal mouse anti-human antibody was used for detection of ER-α (08-1149, Zymed Laboratories, Inc., South San Francisco, California, USA). The Abcam primary antibodies (Abcam, San Francisco, CA, USA) were used as CD34, rat monoclonal. Monoclonal mouse anti-human antibodies were used for detection of PR-antibodies (MA1-410, Affinity Bioreagents Inc. diluted in phosphate buffer solution (PBS) to 1:100). An antigen retrieval procedure was performed for ER-α &PR- A. Sections were pretreated in in 0.01 M sodium citrate buffer (pH 6.0) in a microwave oven for 10 min, and then allowed to cool for a further 20 min. In case of CD34, antigen retrieval was done by trypsin. Specific endogenous peroxidase activity was blocked by treatment with 3% hydrogen peroxide (Segma Technology Inc, Silver Spring MD, Spain) in methanol for 10 min at room temperature. The sections were then washed for 10 min in buffer, and were blocked for 30 min in non-immune horse serum diluted in PBS for ER-α at room temperature. The tissue sections were then incubated with the respective primary antibodies (ER-α; 1:30, PR; 1-100, Cd34; 1-100). Following primary antibody binding, the sections were incubated with the appropriate secondary antibody, for ER-α a biotinylated horse anti- mouse IgG (Vectastain, Vector), diluted in normal horse serum, was used for 60 min at room temperature; in PR-A, incubation was done in a biotinylated goat antimouse (Dako, Glostrup, Denmark). Thereafter the tissue sections were incubated for 30 min at room temperature with a horseradish peroxidase-avidin biotin complex (Vectastain Elite, Vector, CA). The site of the bound enzyme was visualized by the application of 3,3`-diaminobenzidine in H2O2 (DAB kit, Vector, CA). The sections were counterstained with hematoxylin and dehydrated before mounted. Negative control sections were obtained by replacing the primary antibody with non-immune IgG of the equivalent concentration.

Transmission electron microscopy processing:

About 1mm³¬ from uterus, fallopian tube and vagina were fixed in 2.5% solution of glutaraldehyde in 0.1M cacodylate buffer (pH 7.4) for 2 hours and then post fixed for 1-2 hours in 1 % osmium tetra oxide dissolved in the same buffer. Then samples were dehydrated in graded ethanol alcohol, and then embedded in epoxy resin. Semi- thin sections, 0.5 µm, were stained with 1% toluidine blue and examined and photographed by light microscopy. Ultrathin sections, 70-90 nm, were cut and stained with uranyl acetate and lead citrate(Glauret and Lewis, 1998). Specimens were studied by JEOL JEM 1200 EXII Electron Microscope (Jeol Ltd) Research Laboratory, Faculty of Science, Ain Shams University, Egypt.

RESULTS

Light microscopic results:

Examination of H&E-stained sections of control rats showed that the uterine wall was composed of the endometrium, myometrium and

perimetrium. The endometrium was lined with simple columnar epithelium and thick endometrial stroma containing endometrial tubular glands. The myometrium formed of inner circular, middle stratum vascular and outer longitudinal layers of smooth muscles cells (SMCs). In addition, thin connective tissue of perimetrium was seen (Fig.1A). Control fallopian tube showed mucosal folds covered by simple columnar epithelium rested on thin lamina propria. In addition, smooth muscle layer and serosa containing numerous blood vessels were detected (Fig.1B). Vaginal wall was formed of mucosa with stratified squamous epithelium and thick lamina propria, smooth muscle layer and outer adventitia (Fig1C).

Anti ER-α immunostained sections showed that TCs of the uterus exhibited weak immunoexpression in the endometrial stroma and around the glands. TCs were nearly absent from the inner circular smooth muscle layer while few cells were detected around stratum vascular and outer longitudinal layers. TCs were star shaped with many thin long telopodes (Fig.2A). Few TCs were located mainly around longitudinal smooth muscle bundles of myometrium (Fig.2B). In fallopian tube (FT), TCs were detected mainly in the serosa while they were absent from the mucosal folds and muscle layer. Only few spindle shaped positive TCs were seen in the lamina propria. FT serosa showed some TCs adjacent to muscle layer (Fig.2C&D). Vagina showed moderate distribution of TCs in the lamina propria, whereas the positive TCs were seen around and within smooth muscle bundles (Fig.2E&F).

Anti- PR-A immunostained sections showed that TCs were nearly absent from inner circular but numerous in the outer longitudinal SM layers. The inset showed spindle shaped TCs. Uterus showed many immunopositive TCs around the outer longitudinal smooth muscle bundles (Fig. 3A&B). Fallopian tube showed numerous TCs in the mucosal folds, and nearly they were absent from the muscle layer. The inset showed many spindle and pyriform shaped positive TCs in the lamina propria. Higher magnification of FT serosa had few TCs adjacent to muscle layer (Fig. 3C&D). Vagina showed marked distribution of TCs in the lamina propria, specially under the epithelium. Vaginal smooth muscle layer showed numerous TCs on the boundaries of muscle bundles and within the bundles taking the spindle and/ or pyriform shape with long telopodes (Fig. 3E&F).

Anti – CD34 immunostained sections were demonstrated in TCs of endometrial stroma and around the glands, around the SM bundles of outer longitudinal layer of myometrium and few cells were detected around the blood vessels of the perimetrium. TCs were spindle in shape with thin TPs extending from them (Fig.4A&B). FT showed numerous CD34 positive TCs in the lamina propria of mucosal folds and in the serosa. TCs were absent from the muscle layer and appeared at its periphery (Fig. 4C&D). Vagina showed CD34 positive TCs in the lamina propria and around its blood vessels. Vaginal muscle layer had numerous CD34 immunopositive spindle and/ or pyriform shaped TCs located on the boundaries of muscle bundles and within the bundles. TCs were pyriform in shape with oval nuclei and /or spindle shape with elongated nucleus and some of them had thin long TPs. (Fig. 4E&F).

Toluidine blue stained sections of uterine endometrium showed many TCs characterized by small spindle shaped dark nuclei and thin long telopodes encircling the glands and they completely surrounded the branches of spiral arterioles among SM fibers (Fig.5A & B). Fallopian tube (FT) mucosa showed many pyriform shaped telocytes with one telopode mainly under the columnar epithelial lining and in close relation to blood vessels of serosa (Fig.5C &D). Vaginal mucosa contained numerous pyriform shaped TCs with long telopodes under the vaginal epithelium and many spindle shaped TCs with two long thin telopodes in close relation to SMCs and fibroblast in the vaginal musculosa (Fig. 5E& F).

Electron microscopic results:

Electron micrograph of the uterus showed TC with elongated nucleus with a thin band of marginal heterochromatin. TCs had nucleus occupied the whole cell body and telopodes (TP), contained mitochondria, and was seen in close relation to SMCs and collagen fibers (Fig.6A &B). TPs had thin part called podomere and a thick part called podom. Podom contained many mitochondria, rough endoplasmic reticulum, many caveolae in addition to pinched out vesicles extended from the wall of the podoms (Fig.6C).

Telopodes showed a characteristic dichotomous pattern of branching and homocellular junction between their telopodes (Fig. 6D& E).

In fallopian tube, a triangular shaped TC contained a nucleus with a marginal heterochromatin, occupied the whole cell body, and prominent nucleolus could be seen. The cell has three long TPs extended from its angles. One Tp was connected with a SMC with a heterocellular junction. TCs were surrounded by collagen fibers (Fig.7A). Four TCs were also embedded in collagen fibers in close relation to SMCs. TC2,TC3 and TC4 were identified by their characteristic thin and long TPs whereas TC1 was of stellate shape with oval nucleus and prominent nucleolus and also showed convoluted TP. High power magnification of TP had mitochondria and caveolae and thin podomeres. Some shaded vesicles were seen very close to the cell membrane of TCs (Fig.7B- D).

In vagina, TCs were surrounded by SMCs with their characteristic heterochromatic nuclei and telopodes like those present in the FT and uterus (Fig. 8A). TP contained mitochondria and rough endoplasmic reticulum. Mitochondria was seen also in the cell body close to the nucleus. TCs were surrounded by cross sectioned collagen fibers and SMCs. High power magnification showed TCs forming a heterocellular junctions with SMCs by Their cell body (Fig.8B-D).

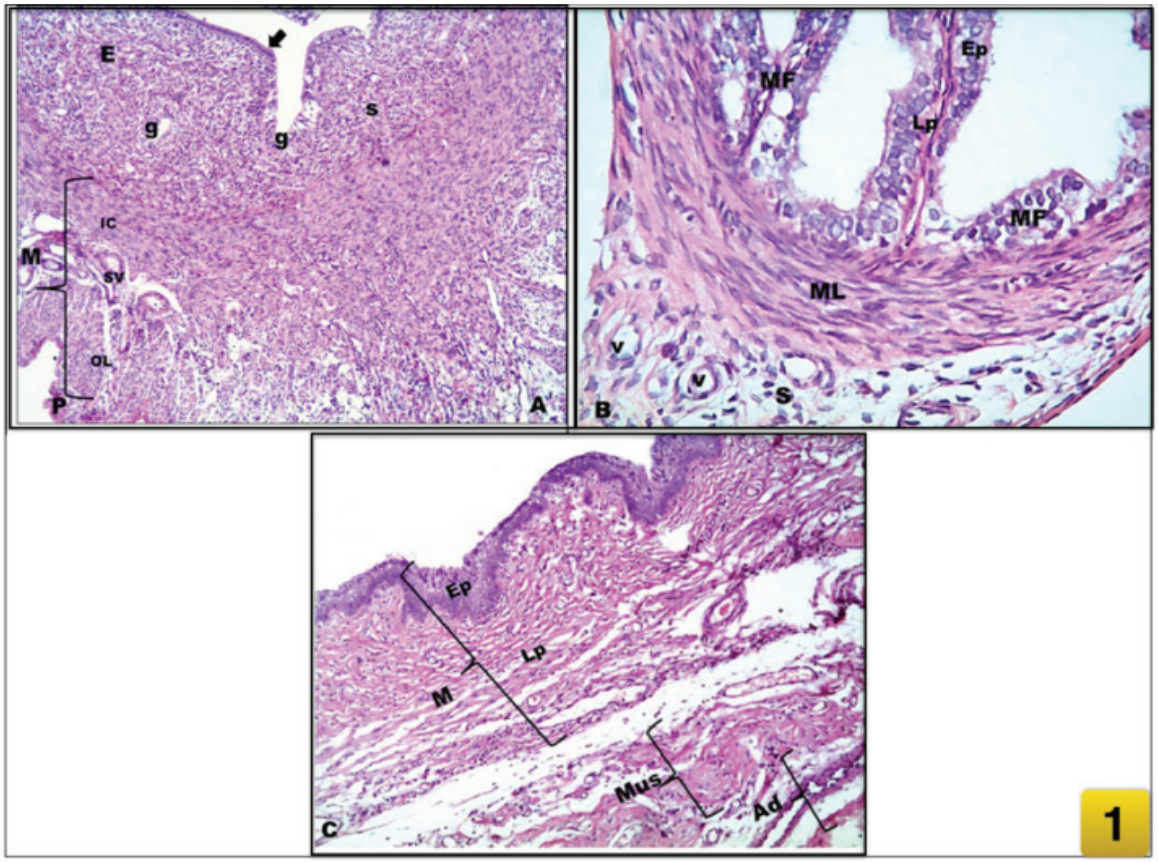

Fig. 1: A photomicrograph sections from control rats show; A) uterus is being formed of endometrium (E) covered with simple columnar epithelium (arrow), and endometrial stroma (S) containing endometrial tubular glands (g). Myometrium (M) is formed of inner circular (IC), middle stratum vascular (SV) and outer longitudinal (OL) layers of smooth muscles, in addition to perimetrium layer (P). B): Fallopian tube with mucosal folds (MF) covered with simple columnar epithelium (Ep) resting on lamina propria (LP) in addition to muscle layer (ML) and serosa(S) containing numerous blood vessels (V). C) Vagina is formed of mucosa (M) with stratified squamous epithelium (EP) and thick lamina propria (LP), smooth muscle layer (Mus) and outer adventitia (Ad).

(H&E stain ; A, C x100 and B x 200)

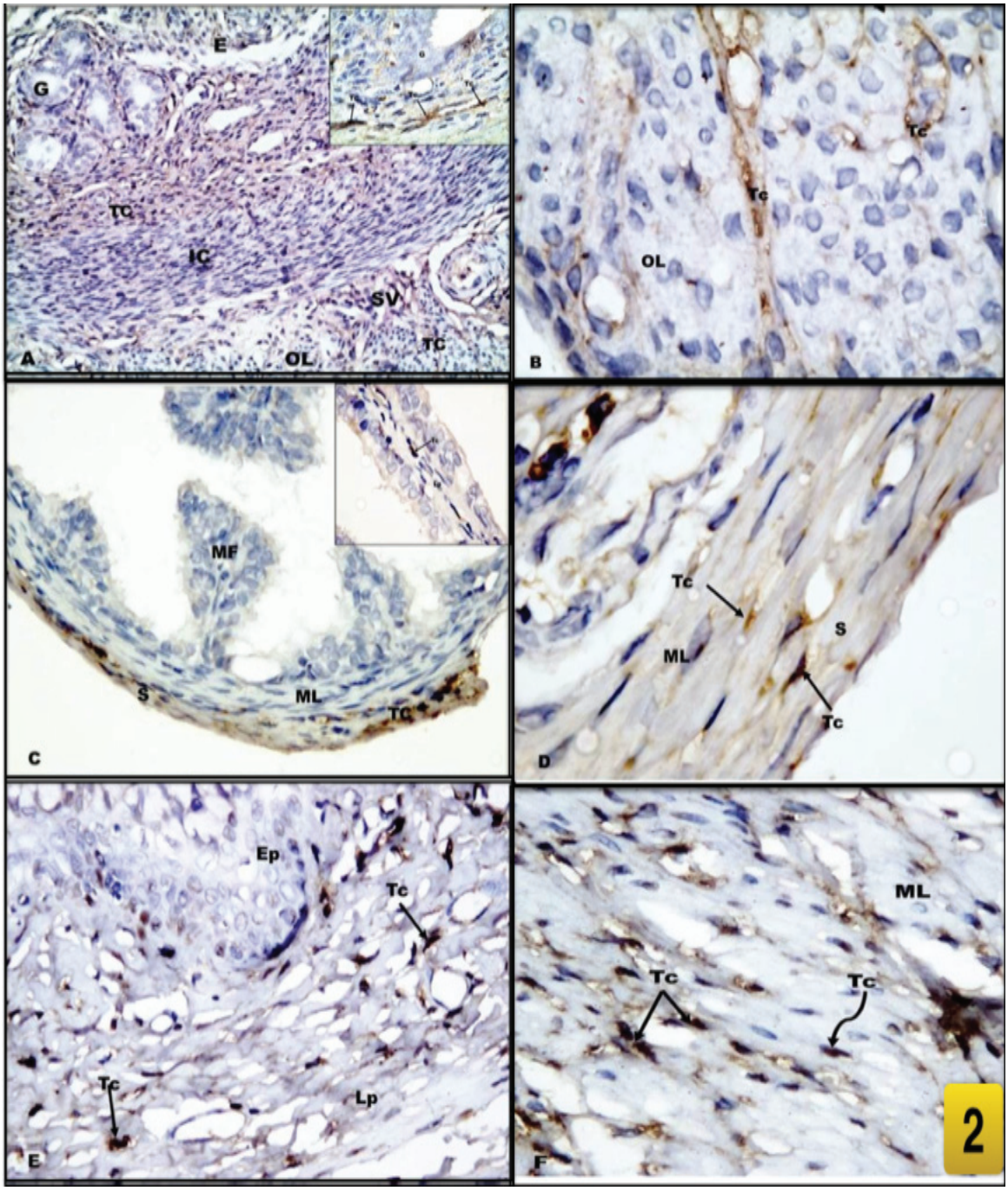

Fig. 2: A photomicrograph sections from control rat show the distribution of ER-α immunopositive telocytes (Tc arrows) in ; A):Uterus has weak ER-α immunoexpression in the endometrial stroma (E) and around the glands (G). Telocytes are nearly absent in inner circular smooth muscle layer (IC), few are detected in stratum vascular(SV) and outer longitudinal (OL) layers. The inset shows immunopositive telocytes (Tc arrows) with many thin long telopodes B): Uterine outer longitudinal smooth muscle outer longitudinal layer (OL) shows few distribution of ER-α immunopositive telocytes (Tc arrows) within the smooth muscle bundles. C): Fallopian tube shows ER-α immunopositive TC in the serosa (S). They are absent from the mucosal folds (MF) and muscle layer (ML).The inset shows few spindle shaped positive telocytes (Tc arrows) in the lamina propria (Lp). D): High power magnification of FT serosa (S) with some telocytes (Tc arrows) adjacent to muscle layer (ML). E) Vagina shows stratified squamous epithelium (EP) and few number of ER-α- positive telocytes (Tc arrows) in the lamina propria (Lp). E) Vagina has numerous telocytes (Tc curved arrows) within the bundles of smooth muscle layer (ML).

(Anti- ER-α immunostain; Ax200 ; B, D, Insets x1000, C, E,Fx400)

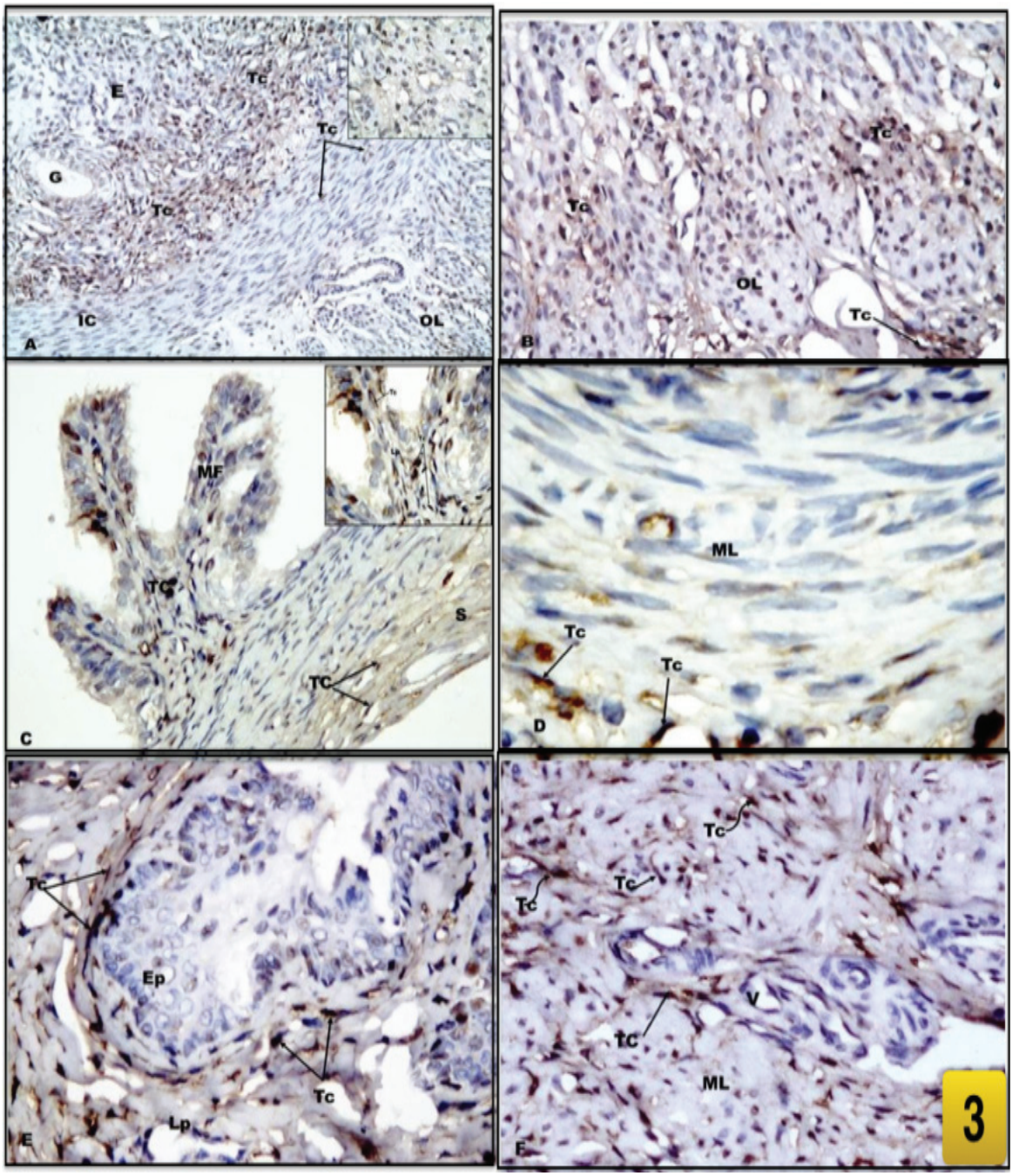

Fig. 3: A photomicrograph sections from control rat show the distribution of PR-A immunopositive telocytes (Tc arrows) in ; A):Uterine endometrial stroma (E) and around the glands (G) with marked expression. TCs are nearly absent from inner circular (IC) but numerous in the outer longitudinal (OL) smooth muscle layers. The inset shows spindle shaped telocytes (Tc arrows). B): Uterus has many immunopositive telocytes (Tc arrows) around the outer longitudinal smooth muscle bundles (OL). C): Fallopian tube has numerous telocytes (Tc arrows) in the mucosal folds, and nearly they are absent from the muscle layer. The inset show many spindle and pyriform shaped positive telocytes (Tc arrows) in the lamina propria (Lp) of mucosal folds. D): High power magnification of FT serosa (S) has few telocytes (Tc arrows) adjacent to muscle layer (ML). E) Vagina has marked distribution of telocytes (Tc arrows) in the lamina propria (Lp) specially under the epithelium (Ep). F) Vaginal smooth muscle layer (ML) shows numerous telocytes (Tc arrows) on the boundaries of muscle bundles and within the bundles (Tc curved arrows) taking the spindle and / or pyriform shape with long telopodes.

(Anti- PR-A immunostain; Ax200; B,C, E, F X400; D and Insets x1000)

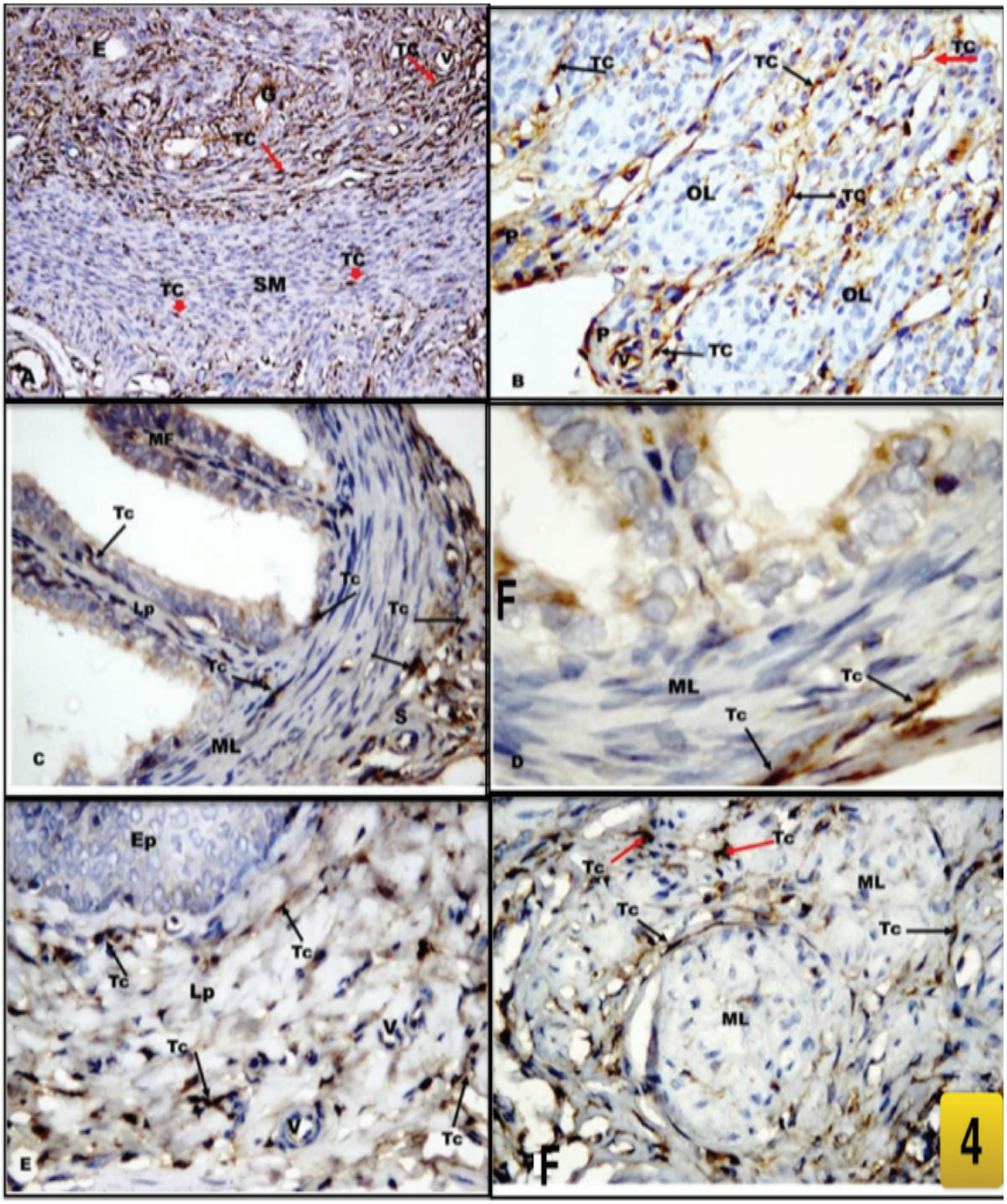

Fig. 4: A photomicrograph sections from control rat showing distribution of CD34 immunopositive spindle shaped telocytes (Tc arrows) in; A):Uterine endometrial stroma (E), around the glands (G) and blood vessels (V) and few cells in the smooth muscle layers (SM). B) Uterine outer longitudinal smooth muscle layer (OL) has telocytes (Tc arrows) around the smooth muscle bundles and few cells are detected around the blood vessels (V) in the perimetrium (P). C): FT has numerous CD34 immunopositive telocytes (Tc arrows) in the lamina propria (Lp) of mucosal folds (MF) and serosa (S). TCs are nearly absent from the muscle layer (ML). D): FT has CD34 positive telocytes (Tc arrow) at the periphery of the muscle layer (ML). E):Vagina has many CD34 positive telocytes (Tc arrows) in the lamina propria (Lp) mainly under the vaginal epithelium (Ep) and around blood vessels (V) taking pyriform with oval nucleus and/ or spindle shape with thin elongated nucleus and some of them have thin long telopodes. F): Vaginal muscle layer (ML) has TCs located on the boundaries of muscle bundles (Tc black arrows) and within the bundles (Tc red arrows).

(Anti – CD34 immunostain; A x200 ; B,C,E,F x400; Dx1000)

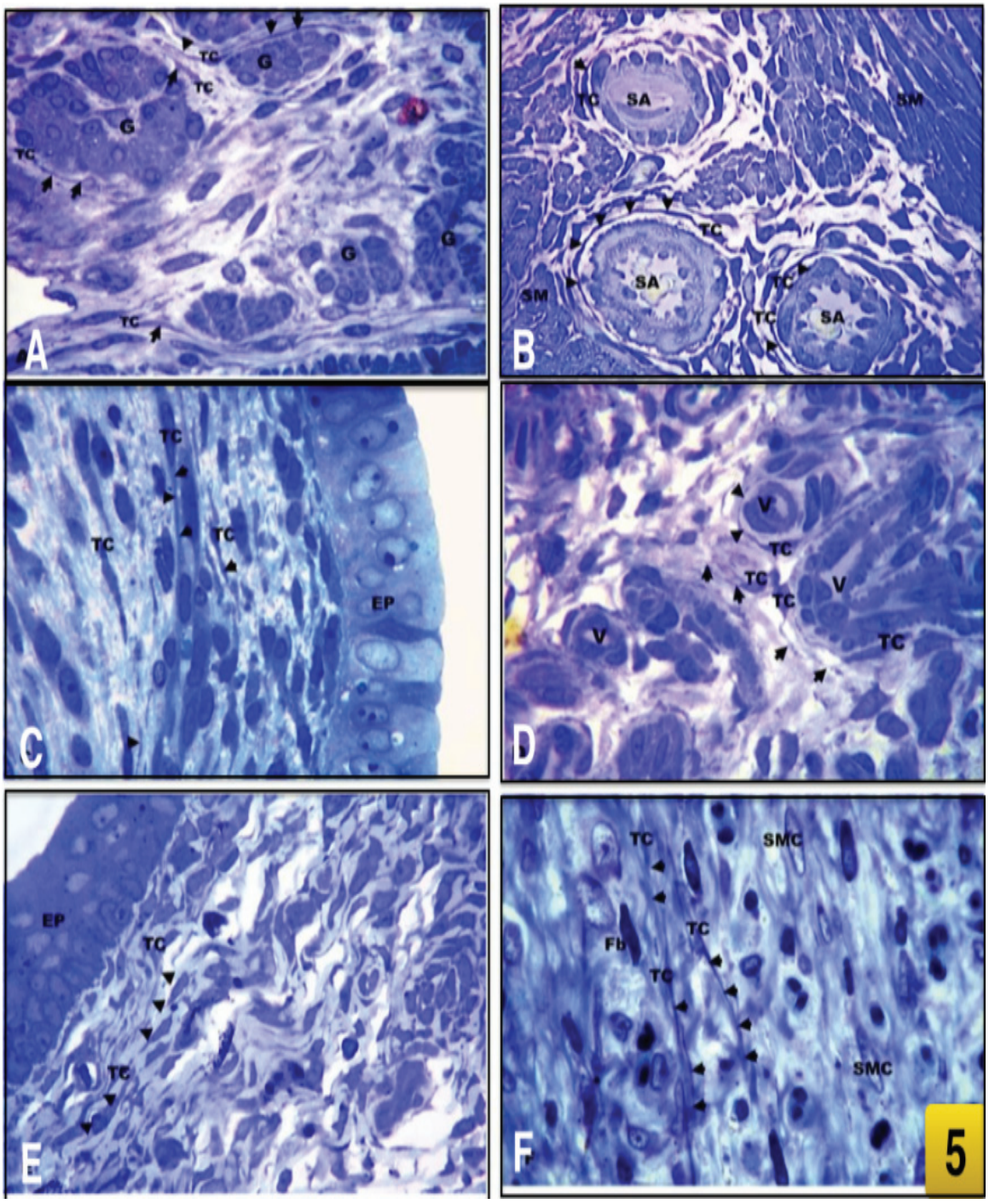

Fig. 5: A photomicrograph of semithin section of control rat showing A):Uterus endometrium with many telocytes (TC) characterized by small spindle shaped dark nuclei and thin long telopodes (arrow heads) encircling the glands(G).B) Uterine myometrium with branches of spiral arterioles (SA) among smooth muscle fibers (SM) are completely surrounded by thin and long telopodes (arrow heads) of many telocytes (Tc). C): Fallopian tube mucosa showing simple columnar epithelium and many pyriform shaped telocytes (Tc) with one telopodes (arrow heads). D): Fallopian tube serosa showing some telocytes (Tc) in close relation to blood vessels (V) also taking the pyriform shape with characteristic one telopode (arrow heads). E) Vaginal mucosa with numerous pyriform shaped telocytes (Tc) with long telopodes (arrow heads) located under the vaginal epithelium (Ep). F): Vaginal musculosa showing many spindle shaped telocytes (Tc) that have two very long thin telopodes in close relation to smooth muscle cells (SMC) and fibroblast cells (Fb).
(Toluidin blue stain; A- F x1000)

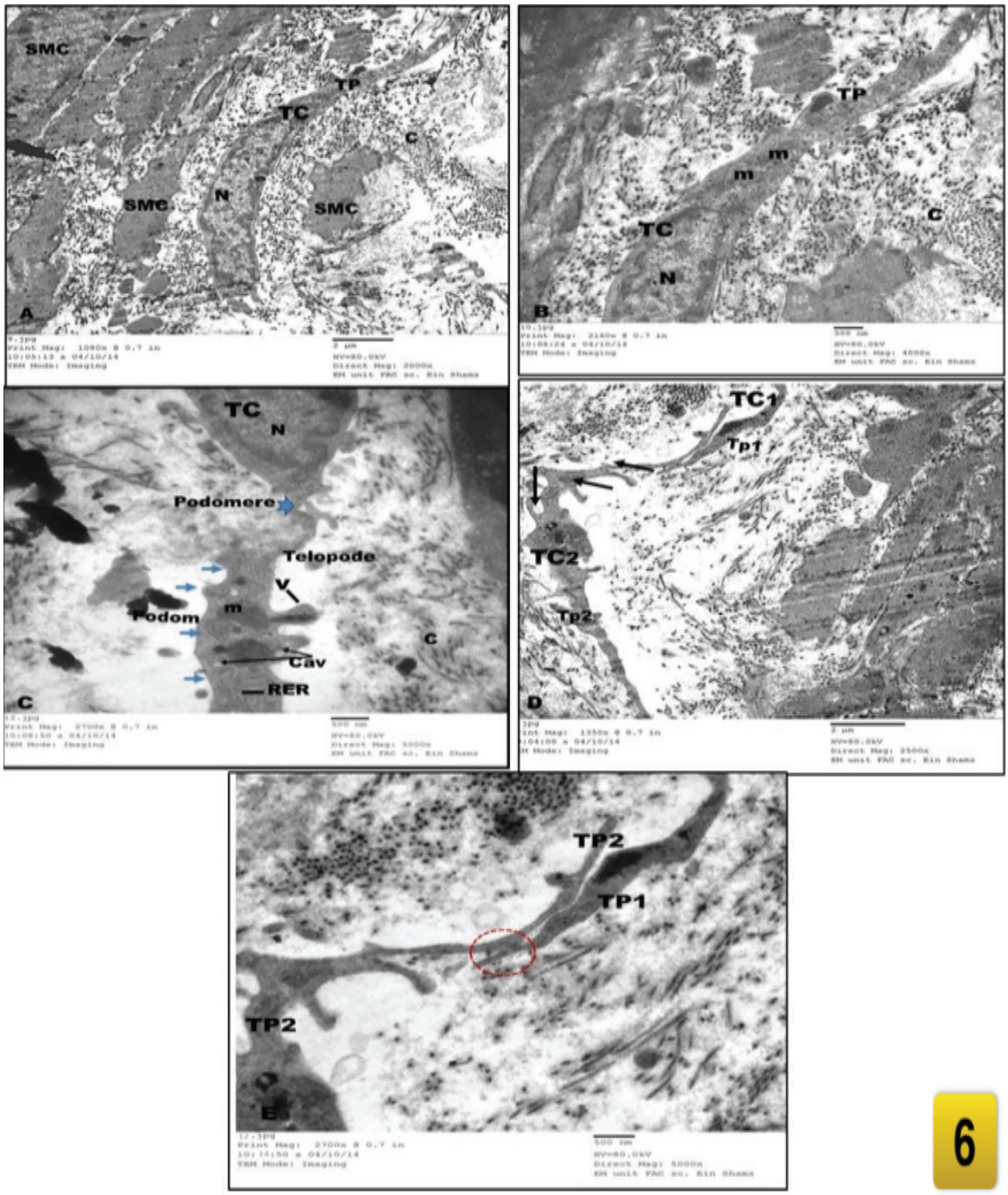

Fig. 6: An electron micrograph of control rat uterus show; A): A Telocyte (TC) has elongated nucleus (N) occupying the whole cell body and has a thin band of marginal heterochromatin. There is one telopode (TP) extends from the cells. TC is seen in close relation to smooth muscle cells (SMC) and collagen fibers(C). B): High power magnification of the previous figure showing part of telocyte (TC) with nucleus (N) and telopode contains mitochondria (m). Collagen fibers (C) can be seen. C):A part of another telocyte (TC) showing nucleus occupying the whole cell body and a telopode has thin part called podomere and a thick part called podom. Podom contains many mitochondria (m), rough endoplasmic reticulum (RER), many caveolae (Cav) and pinchedout vesicles are seen extending from the wall of the podom. D): Tow telopodes (TP1 and TP2) of two telocytes (TC1 and TC2) which are in contact with each other. TP2 shows dichotomous pattern of branching (arrows). E): High power magnification of figure (D) shows homocellular junction (red circle) between the two telopodes (Tp1and Tp2).

(TEM, Ax2000, Bx4000, Cx5000, Dx2500 & Ex5000)

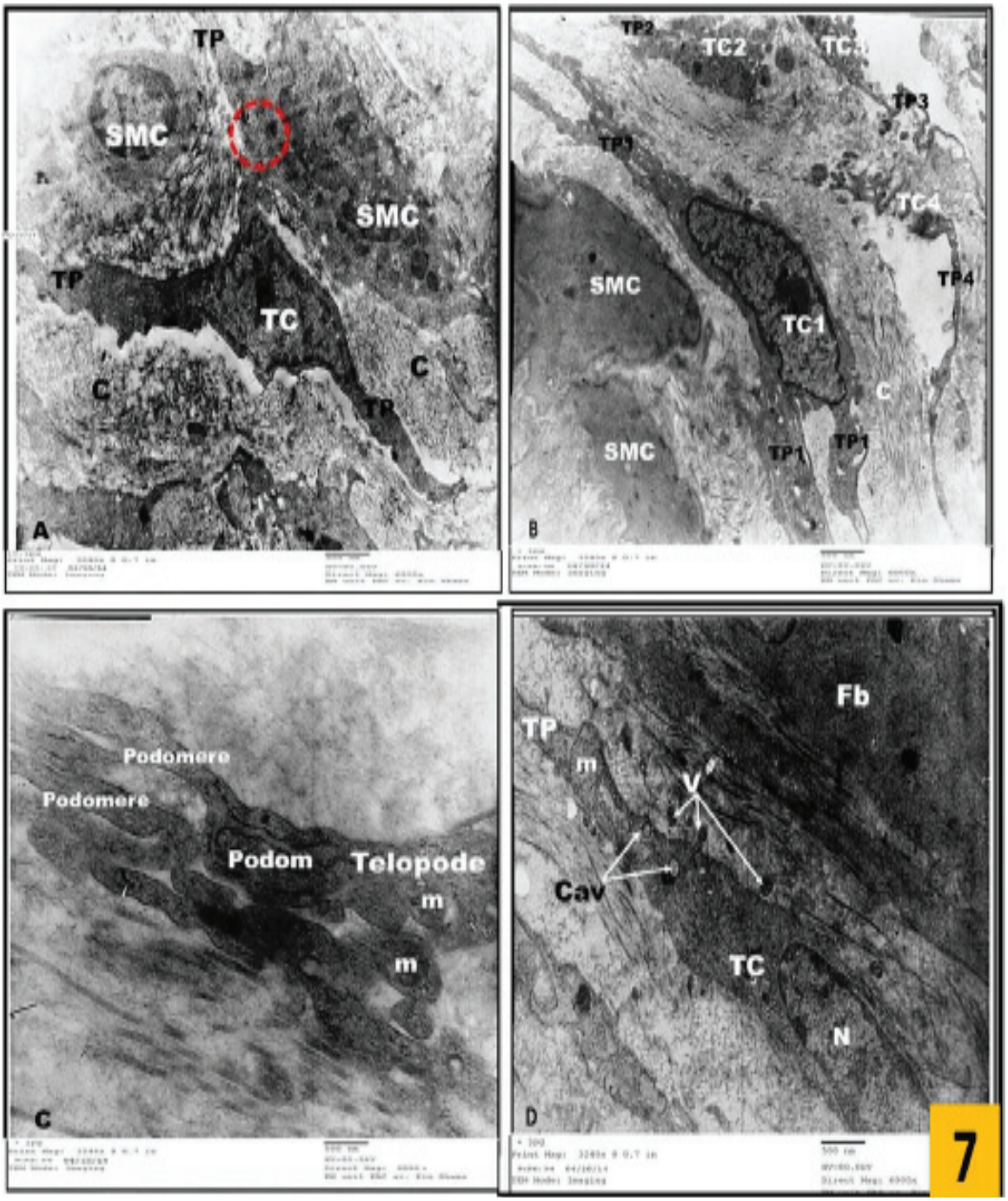

Fig. 7: An electron micrograph of control rat fallopian tube shows, A): A triangular shaped telocytes (TC) containing nucleus, with marginal heterochromatin, occupying the whole cell body and shows prominent nucleolus. The cell has three long telopodes (TP) extending from its angles. One telopodes comes in close contact with a smooth muscle cell (SMC) forming a heterocellular junction (red circle). Telocyte is surrounded by collagen fibers (C). B): Four telocytes embedded in collagen fibers (C) adjacent to smooth muscle cells (SMC). Telocyte1(TC1) is of stellate shape with oval nucleus and prominent nucleolus and showes convoluted telopodes (TP1). TC2,TC3 and TC4 are marked by their characteristic thin and long telopodes (TP2,TP3&TP4). C) High power magnification of fig. (B) shows a typical telopode which appears long and highly convoluted. Note a large podom containing mitochondria (m) and thin podomeres. D): Apart of Telocyte (TC) containing nucleus (N) and has telopode(TP) containing many mitochondria (m) and caveolae (Cav). Some shed vesicles (v) are seen very close to the cell membrane of telocyte in addition to adjacent fibroblast (Fb).

(TEM; A,B, D x6000 & C x8000)

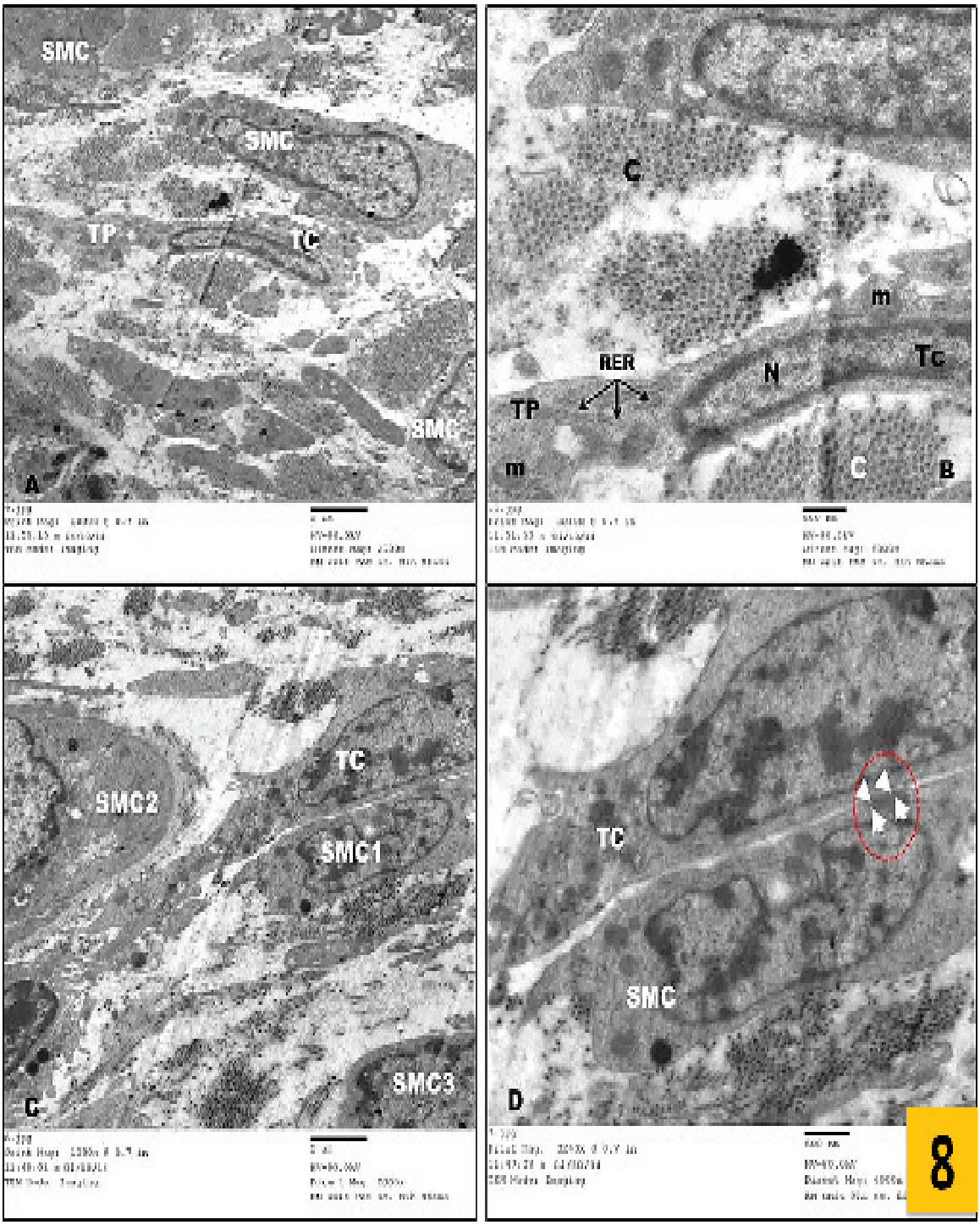

Fig. 8: An electron micrograph of control rat vagina showing A): Telocyte (Tc) surrounded by many smooth muscle cells (SMC) and identified by its elongated nucleus (N) and its characteristic telopode (TP). B): High power magnification of fig.(A) shows telocytes (TC) with a telopode (TP) containing mitochondria (m) and rough endoplasmic reticulum (RER). One mitochondria is seen in the cell body close to the hetrochromatic nucleus (N). TC is surrounded by cross sectioned collagen fibers (C). C): Telocyte (TC) is surrounded by three smooth muscle cells (SMC1,2&3).TC comes in close contact with SMC1. D): High power magnification of fig.(C) shows teloycte (TC) forming a heterocellular junction (red circle and arrow heads) with SMC1 by its cell body.

(TEM; A&Cx 2000, B&Dx6000).

DISCUSSION

Telocytes form a remarkable new cell species found in many types of tissue. They were discovered by (*Popescu et al., 2005b*). Our study was intended to discuss deeply the terms of Telocyte (TC) and Telopodes (Tp) in female genital tract through ultrastructural and immunohistochemical expression of ER-α, PR – A and CD34.

In our study, TCs exhibited weak ER-α and marked PR-A immunoexpression in the endometrial stroma and around the glands. TCs were nearly absent from the inner circular smooth muscle layer while few cells were detected around stratum vascular and in the outer longitudinal muscle bundles of myometrium. This finding goes with that of (*Nagla, 2012*) who observed few scattered TCs in the myometrium and the cells were mainly scattered around the glands. Similarly Hatta *et al.* (2012) reported that there are TCs in the endometrial stroma of the stratum functionalis and in the stromal space between the gland. In comparison between estrogen and progesterone immunoexpression, Popescu *et al.* (2005 a,b) and Cretoiu *et al.* (2006) mentioned that the PR-A expression was stronger than for ER which was either weakly positive or completely negative. In vitro study proved that TCs can express steroid hormone receptors and could also be 'hormonal sensors' in human uterus, since there is evidence of some uterine stromal cells that play a role in endometrial growth and differentiation in a hormone-dependent manner (*Cretoiu et al.,2006; 2012b*).

Moreover, CD34 immunopositive TCs were detected mainly in a similar area like PR-A in addition to its presence around the blood vessels of perimetrium. Distribution of CD34 in TCs were appeared to be of strategic location specially around the glands. This pattern of distribution could raise the suggestion that TCs may support the structure of the gland and the nearby stroma by forming a scaffold around them (*Popescu et al., 2005a,b; Cretoiu et al., 2006*).

Toluidine blue stained sections of uterine endometrial glands showed many TCs characterized by small spindle shaped dark nuclei and thin long telopodes which encircled branches of spiral arterioles of smooth muscle layer of myometrium. Further examination of TCs using electron microscope showed elongated nuclei which contained a thin band of marginal heterochromatin attached to the nuclear envelope. The nucleus was occupying the whole cell body. In addition, one telopodes (TPs) extending from the cells. The TCs were seen in close relation to smooth muscle cells and collagen fibers. In agreement with the present study, Popescu *et al*., (2005b and 2006) mentioned that the TCs represented approximately 7% of the total cell number on random semi-thin myometrial tissue sections. They added that TCs appeared as small spindle-shaped cells with one or two thin long processes named telopodes up to 100 micrometers long and only 20–200 nanometers wide.

In this study, uterus, fallopian tube and vagina revealed TPs consist of long thin tubes called podomers interspersed with short dilations called podoms. Previously mentioned results were in accordance with that described by (Cretoiu *et al*., 2012b ; 2013). He and his colleagues described the appearance of TPs by the appearance of axonal boutons.

By EM, this study revealed that TCs of the uterus and vagina have slender shaped cell body with a thin rim of cytoplasm surrounding the nucleus and extremely long and thin tubular processes. Our results were in agreement with(Cretoiu *et al*., 2012b) who reported that the TCs have a small cell body with a scarce amount of cytoplasmic organelles surrounding the nucleus; e.g. mitochondria 3% and endoplasmic reticulum 1–2% of cell volume. The cell body shape depends on the number of Tps, but usually it is spindle or triangular in shape.

In this study, many caveolae and pinchedout vesicles were seen extending from the wall of the podom. Cretoiu *et al*., (2013) mentioned that TC might act as mechanoreceptors and it had been detected at the border of smooth myometrial fibers and also among them. Therefore, it justifies the presumption that they might be capable of detecting and translating stretch information to the nucleus. Then it activates genes responsible for protein synthesis. The myogenic uterine contractility modulation under hormonal control could involve TCs either by transferring

bioactive molecules or by direct stimulation of myocytes.

The mechanism by which the myometrial contractility is initiated in a coordinated fashion is still a subject of debate. Uterine myocytes contract via excitation–contraction coupling based on detailed study of cellular mechanisms (Taggart *et al*., 2012). However, the initiation of electrical signals and the coordination of contractions throughout the organ are, to some extent, unknown.

In this study, immunohistochemichal expression of TC for ER-α , PR-A and CD34 in the fallopian tube (FT) were more numerous in the mucosal folds among the columnar epithelium and in the lamina propriae but were few in the periphery of the muscle layer and in the serosa. These data were in agreement in part with Cretoiu *et al.,* (2012b) who reported that the distribution of TCs decreases from the sub-epithelial tissues towards the serosa and it takes a pyriform shape. A similar distribution of TCs was also observed by (Cretoiu *et al.,* 2012a) who found that TCs were more numerous in fallopian tube lamina propria and in between smooth muscular fibers with the following percentage; area in the lamina propria found close to basement membrane (18±2%); area containing the entire lamina propria thickness (~8%); muscularis (7.8±1.2%) and the remaining zone beneath serosa.

Giretti and Simoncini (2008) and Tetel *et al*. (2009) described two types of receptors in the fallopian tubes; ER-α and β, and PR- A and B for each one of steroid hormones in a cell cultures enriched in TCs. It was proved that the sex hormones estrogens and progesterone perform their action through the nuclear receptors and act as ligand regulating transcription factors as well as by membrane-associated receptors and signaling cascades and therefore were involved in the regulation of many functions, including reproduction, especially in ovum fertilization and embryo implantation (*Popescu et al., 2005a*).

In this work, FT mucosa showed many pyriform shaped TCs with one telopode mainly under the columnar epithelial lining and in close relation to blood vessels of serosa. Moreover, in ultrathin sections of fallopian tube and vagina, a triangular shaped TC contained a nucleus with a marginal heterochromatin, occupied the whole cell body, and prominent nucleolus could be seen. The cell has three long TPs extended from its angles. One Tp was connected with a SMC with a heterocellular junction. These results were similar to those given by Cretoiu *et al.* (2012 a, b) who added that TC had different cellular bodies shapes which may be pyriform; (having only one prolongation), spindle (with two opposite prolongations), triangular and other shapes with more than three prolongations .

Yang *et al.* (2014) added that TCs usually detected among bundles of collagen and elastic fibers being connected in the components of the interstitium or combined a different structural an integrate system with their extremely long and thin Tps with the formation of an organized and unique 3-D extracellular matrix of the connective tissue within organs. Therefore, damage or loss of TCs might disturb their spatial relationships with adjacent multicellular entities, which finally causes oviduct dysfunction and consequent reproductive problems.

In our study, examination of vagina by immunohistochemistry revealed that TCs showed moderate distribution of anti ER-α immunostained and marked distribution of anti PR-A Regarding the relation between estrogen and progesterone during estrus cycle, progesterone is known to be high in proestrus, then drastically drop in estrus, followed by slow rise in metestrus and finally its level starts to rise reaching high concentration during diestrus phase (Staley and Scharfman, 2005). The previous explanation can ensure our immunohistochemical variation seen in the vaginal samples which were taken in diestrus phase. Other scientists added that, it is believed that high level of progesterone (diestrus phase) within the tissue would inhibit vaginal contractions, while low levels (estrus, metestrus) allow spontaneous contractions (Griffiths *et al.,* 2006; Fernanda *et al.,* 2014).

In animal models for the study of vaginal function during sexual behaviors and in initiation of the urethrogenital reflex, Munarriz et al. (2003) described phasic vaginal contractions which were attributed to pacemaking within the smooth muscle layer or autonomic nervous

activity. Others suggesting pacemaking within the SM to be ICC (*Giraldi et al., 2002*).

In this research, TC was surrounded by SMCs with its characteristic heterochromatic nucleus and telopodes like those present in the FT and uterus. TPs contained mitochondria and rough endoplasmic reticulum. Mitochondria was seen also in the cell body close to the nucleus in addition, TCs formed a heterocellular junction with SMC by its cell body. This ultramicroscopic structure was similar to what was given by many scientist in the field of studying TCs Cretoiu *et al.* (2012a,b); Yang *et al.*(2014) but no one of them mentioned their structure in the vagina.

In conclusion, TCs showed weak positive ER-α and marked expression of PR-A and CD34 immunoexpression in the endometrial stroma and around the glands, in stratum vascular and outer longitudinal muscle layers of the uterus, serosa of the fallopian tube and in the lamina propria and the boundaries of smooth muscle bundles and within the bundles of the vagina. Ultramicroscopically, triangular or stellate-shaped TCs in uterus, fallopian tube and vagina had elongated nucleus occupying the whole cell body, thin band of marginal heterochromatin and prominent nucleolus, one or more telopodes (TPs) with dichotomous pattern of branching. TPs had thin podomere and thick podom. Podom contains many mitochondria, rough endoplasmic reticulum (RER), many caveolae and pinchedout vesicles. TCs had either homocellular junction between TCs or heterocellular junction between them and smooth muscle cells. TCs were surrounded by collagen fibers. The presence of steroid hormone receptors suggests that TCs could behave as a sensors controlling the peristalsis or explain infertility in patients without any proven abnormalities. Therefore this study looked to produce baseline information on TCs in female genital tract and represent the first comprehensive description of TCs in the vagina.

CONCLUSION

TCs are new type of cells with special ultrastructure and immunophenotype characters that are detected in the female genital tract.

RECOMMENDATION

Further studies on TCs distribution in the female genital tract during different stages of estrus cycle and correlation between their distribution and their role in peristalsis of fallopian tube and in new approach controlling infertility or myometrial contraction during pregnancy.

CONFLICTS OF INTEREST:

There are no conflicts of interest.

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خصائص التركيب الدقيق والتوزيع المناعي لخلايا التيلوسيت في الجهاز التناسلي لإناث الجرذان البيضاء غير الحوامل

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ملخص البحث

المقدمة: إن المعلومات المتوفرة عن خلية التيلوسيت، والتي تم اكتشافها في السنوات الأخيره قليلة جدا.

الهدف: تم تصميم هذه الدراسة لإعطاء فكره نسيجية، نسيجية كيميائيه مناعية، والتركيب الدقيق لتوزيع خلايا التيلوسيت في الجهاز التناسلي للإناث.

مواد وطرق البحث: تم اخضاع عدد ثلاثين جرذ أبيض لفحص مسحة من المهبل مأخوذه من ثلاثين جرذ خلال منتصف دورة الحيض. تم الحصول على عينات من الرحم، قناة فالوب، المهبل وتجهيزهم لكل من الميكروسكوبين الضوئي والالكتروني.

النتائج: أظهرت خلايا التيلوسيت تفاعل ايجابى ضعيف لمستقبلات الإستروجين-ألفا، وتفاعل مناعي ملحوظ لمستقبلات البروجيستيرون -ألفا ، سى دي 34 فى الطبقة الطلائيه للرحم وحول خلايا الرحم، وطبقة الأوعية الدموية، وكذلك طبقة العضلات الناعمة الطولية الخارجية للرحم. كما يوجد التفاعل فى الطبقة الخارجية لقناة فالوب بالإضافة الى طبقة الصفيحة المخصوصة وفى النسيج الضام الموجود تحت الغشاء الطلائي للمهبل. أظهر التركيب الميكروسكوبي الدقيق للرحم، وقناة فالوب، المهبل أن خلايا التيلوسيت لها شكل نجمي أو مثلثي وتحتوي على نواه طولية تحتل كل جسم الخلية ولها شريط رفيع من الكروماتين الطرفي المختلف ونوية ظاهره بوضوح. وللخلية زوائد جانبية ومتفرعة بشكل مزدوج تحتوي على بودومير رفيع ، وبودوم سميك. هذا و يحتوي البودوم السميك على العديد من الميتوكوندريا، الشبكة الإندوبلازمية الخشنة، والكثير من الكهوف والحبيبات المتفرعة منها. وترتبط خلايا التيلوسيت مع بعضها البعض بروابط خلوية متشابهة أوترتبط مع الخلايا العضلية الناعمة القريبة منها بروابط مختلفة.وتحاط خلايا التيلوسيت بألياف الكولاجين.

الخلاصة: خلايا التيلوسيت هي نوع جديد من الخلايا والتي لها تركيب دقيق خاص بها وخصائص للشكل المناعي وتوجد هذه الخليه في الجهاز التناسلي للأنثى. ولهذا فاننا بحاجة للمزيد من الدراسة لتوزيع هذه الخلايا في الجهاز التناسلي للأنثى خلال الفترات المختلفة لدورة الحيض، كما أنه يجب أن نحاول أن نجد العلاقة بين توزيع خلايا التيلوسيت ودورها في التحكم في العقم أو في انقباض عضلات الرحم أثناء الحمل.