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# Telocytes in Human Fallopian Tube and Uterus Express Estrogen and Progesterone Receptors

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## 1. Introduction

Evidence has accumulated over a number of years for the existence of a new cell type found in cavitary and parenchymatous organs - called telocytes (TCs). The cell biology of TCs, and especially their function is a rapidly growing area of biomedical research (Figure 1) (free-access data is available at [www.telocytes.com](http://www.telocytes.com)). TCs are also present in fallopian tube (Popescu et al., 2005a) and uterine walls (Ciontea et al., 2005).

Progress in cellular and molecular techniques led to the identification of subtypes and isoforms of estrogen receptors (ER) (Green et al., 1986; Kuiper et al., 1996; Tremblay et al., 1997) and progesterone receptors (PR) (Kastner et al., 1990; Giangrande & McDonnell, 1999) in the female reproductive tract, two for each receptor (ER $\alpha$  and  $\beta$ , and PR A and B). Cells of the female reproductive tract are subject to hormonal control via sex steroids receptors. Subsequently, we investigated the expression of estrogen receptor (ER) and progesterone receptor (PR) in cell cultures enriched in TCs, obtained from the muscle coat of both the fallopian tube and uterus.

## 2. The concept of telocytes

In 2005, we described a new cell type which we called interstitial Cajal-like cells (ICLC) due to their similarity with canonical gastrointestinal interstitial cells of Cajal (ICC). By using electron microscopy, immunohistochemistry and cell cultures, we revealed that ICLC have particular features that distinguish and separate them from the ICC and/or other interstitial cells. Given these new findings, Popescu renamed ICLC to TELOCYTES (TCs) (Popescu & Faussone-Pellegrini, 2010) by using the Greek affix 'telos', meaning "goal", "end", and "fulfilment", suggesting cells with a particular goal, accomplished through their extremely long prolongations. The new term aims to avoid any confusion between these cells and other interstitial cells such as fibroblasts, mesenchymal cells, and myofibroblasts. The very long and thin prolongations emitted by TCs were re-defined as telopodes (TPs). TPs are built of alternating thin segments known as podomers ( $\leq 200$  nm, below the resolving power of light microscope) and dilated segments called podoms (with a mean width of 462.31 nm), which accommodate mitochondria, rough endoplasmic reticulum and caveolae.

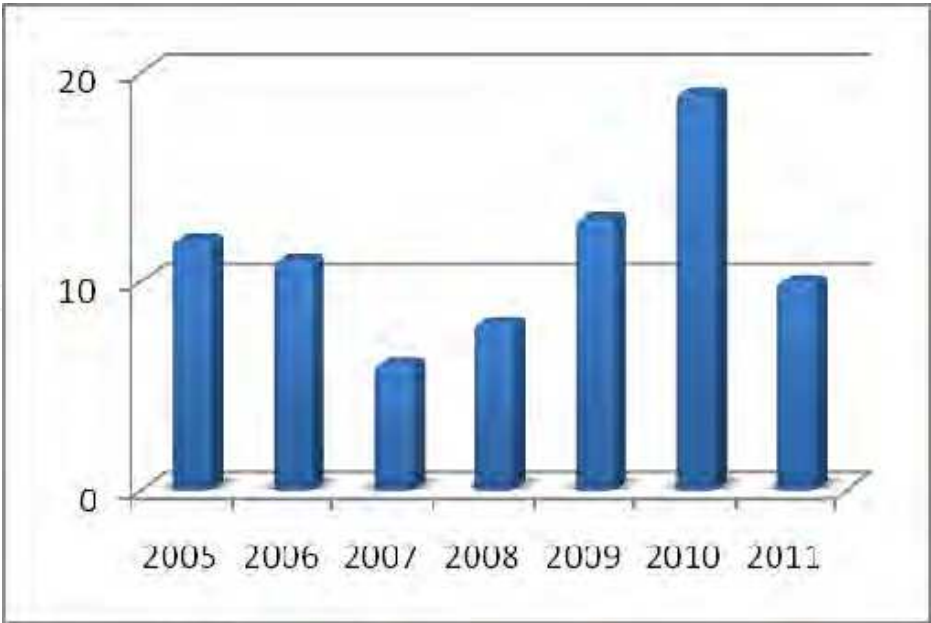


Fig. 1. Graph showing the ascending trend of the number of articles retrieved from [www.pubmed.gov](http://www.pubmed.gov) using key words “telocytes”, “interstitial Cajal-like cell”, or “ICC-like”. The number of published papers is increasing exponentially.

TPs are a distinctive feature of TCs and are characterized by the following main features:

- **Number:** can vary between 1 and 5. Frequently, only 2-3 telopodes are observed on a single section, depending on site and angle of section (Figure 2, 3), since their 3D convolutions prevent them from being observed at their full length in a very thin 2D section (Figure 4);

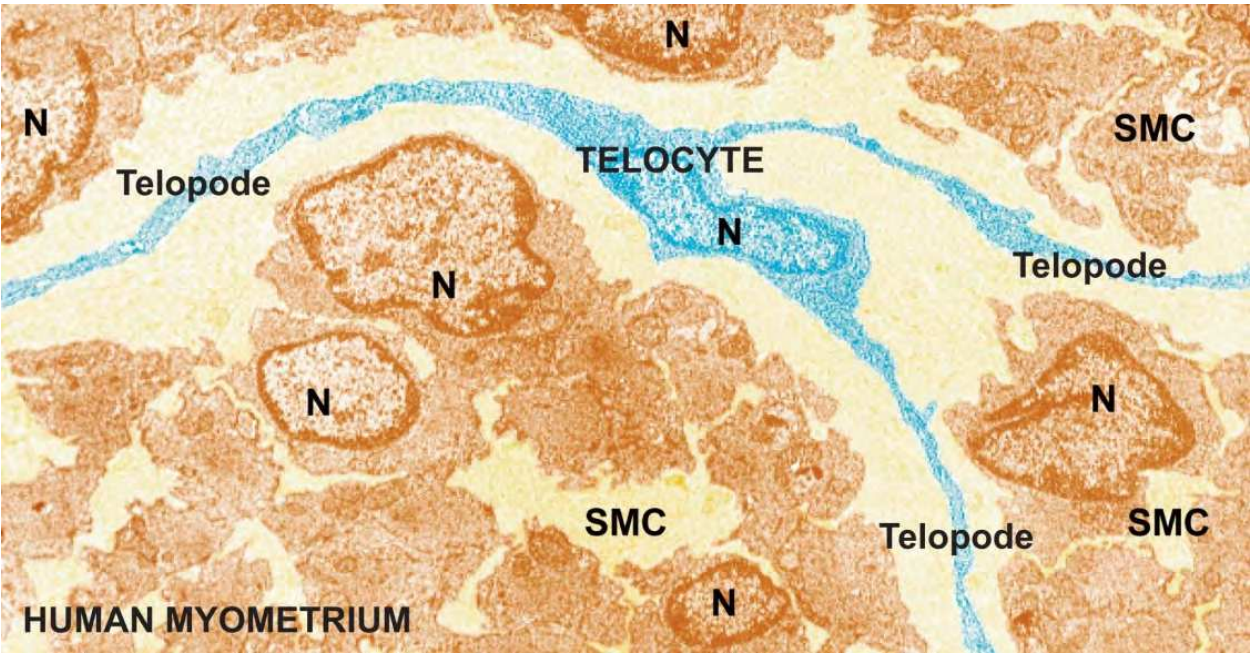


Fig. 2. Non-pregnant myometrium. Digitally coloured TC (blue) with 3 TP that encircle bundles of cross-cut smooth muscle cells (SMC, Sienna brown); N - nuclei. Reproduced, with permission, from Ciontea et al., 2005.



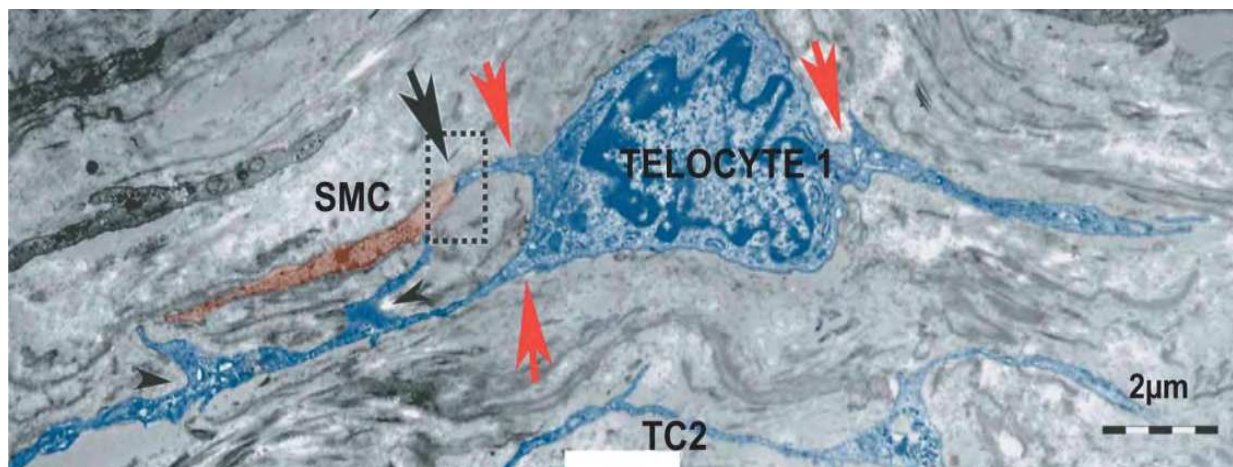


Fig. 3. Human term placenta. Telocyte 1 (blue) has few organelles in the perinuclear area and three emerging TPs (red arrows); black arrowheads mark the dichotomic branching points. Note the podoms and podomeres. The black arrow indicates the junction between TPs and a smooth muscle cell (SMC, coloured in brown). Reproduced, with permission, from Suciú et al., 2010.

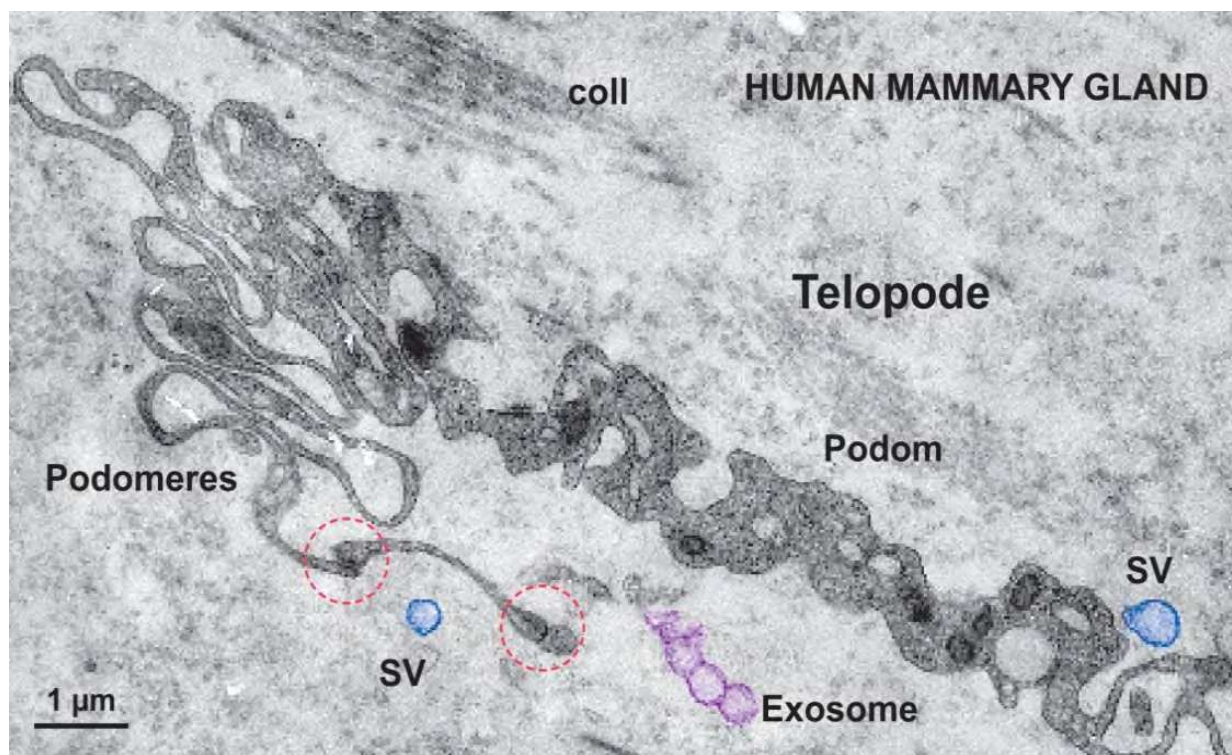


Fig. 4. Human resting mammary gland stroma. One TC hallmark, namely TPs, appears quite long and convoluted. Note homocellular junctions marked by red circles, as well as shed vesicles (blue) and an exosome (violet). Reproduced, with permission, from Gherghiceanu & Popescu, 2005.

- **Length:** tens to hundreds of  $\mu\text{m}$ , as measured on EM images (Figure 5). However, under favorable cell culture conditions, their entire length can be captured in several successive images (Figure 6);

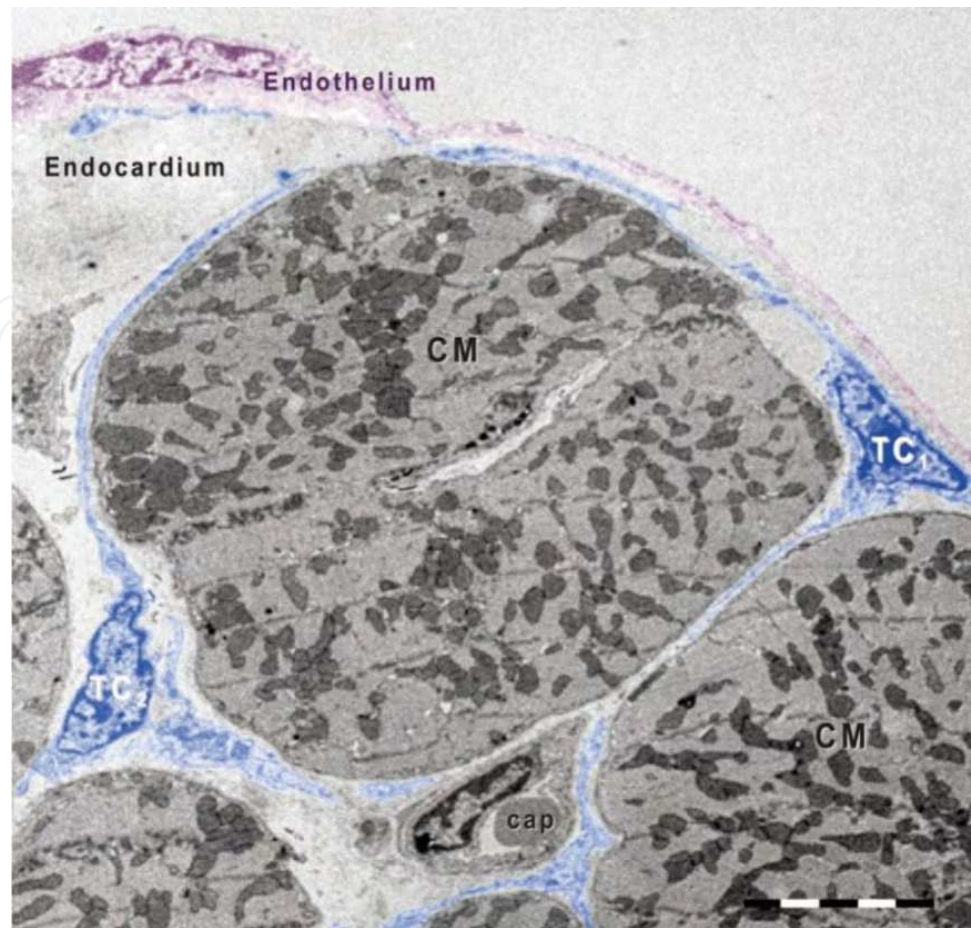


Fig. 5. Digitally coloured electron micrograph of mouse ventricular endocardium (burgundy). TCs (blue) form an interstitial network in the heart. A subendocardial telocyte (TC<sub>1</sub>) sends TP between cardiomyocytes (CM) and communicates with TC<sub>2</sub>. Cap, blood capillary. Scale bar 5  $\mu$ m. Reproduced, with permission, from Gherghiceanu et al., 2010.

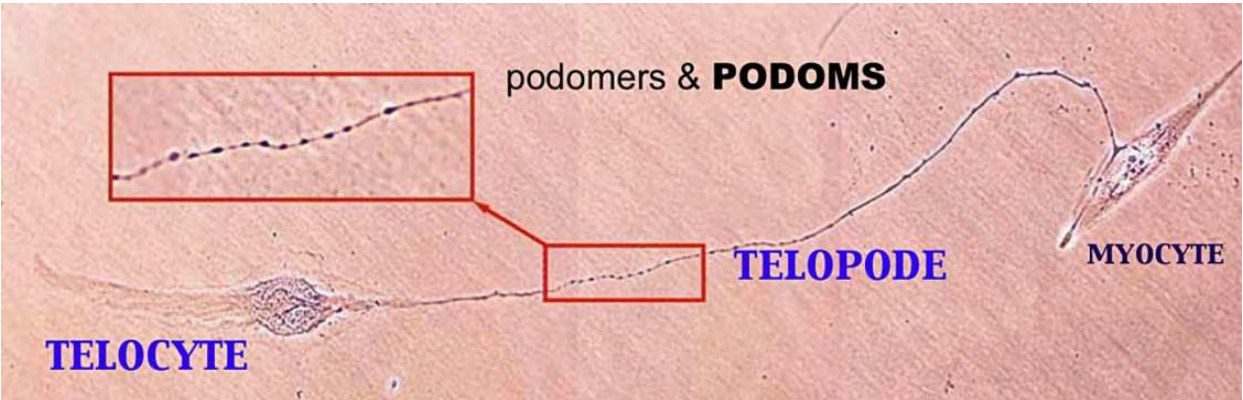


Fig. 6. Non-pregnant human myometrium in cell culture, day 3, the first passage. Giemsa staining. TC establishing contacts with a myocyte by a TP of about 65  $\mu$ m long. Photographic composition of 4 serial phase contrast images; original magnification 40x. A higher magnification of TP (rectangles) clearly shows a moniliform aspect: at least 40 specific dilations (podoms) connected by thin segments (podomers) are visible in a 'beadlike' fashion. Reproduced, with permission, from Ciontea et al., 2005.



- **Thickness:** uneven caliber, mostly below  $0.2\ \mu\text{m}$  (below the resolving power of light microscopy), visible under electron microscopy;
- **Moniliform aspect:** podoms and podomeres (Figures 7, 8); average caliber of podomeres:  $0.1\ \mu\text{m} \pm 0.05\ \mu\text{m}$ , min. =  $0.003\ \mu\text{m}$ ; max. =  $0.24\ \mu\text{m}$ ; Podoms accommodate: mitochondria, (rough) endoplasmic reticulum, caveolae, a trio called 'Ca<sup>2+</sup>-uptake/release units' (Figure 9);

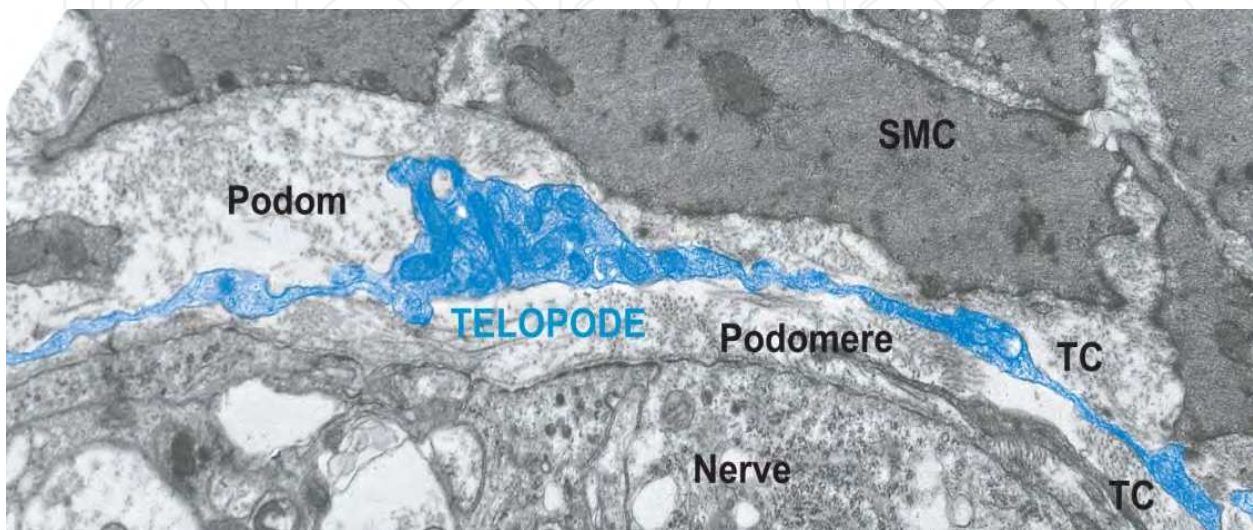


Fig. 7. Rat jejunum. A typical TP (blue) located between smooth muscle cells (SMC) and nerve endings. Note a large podom and the corresponding podomeres. TC body is not captured in the image.

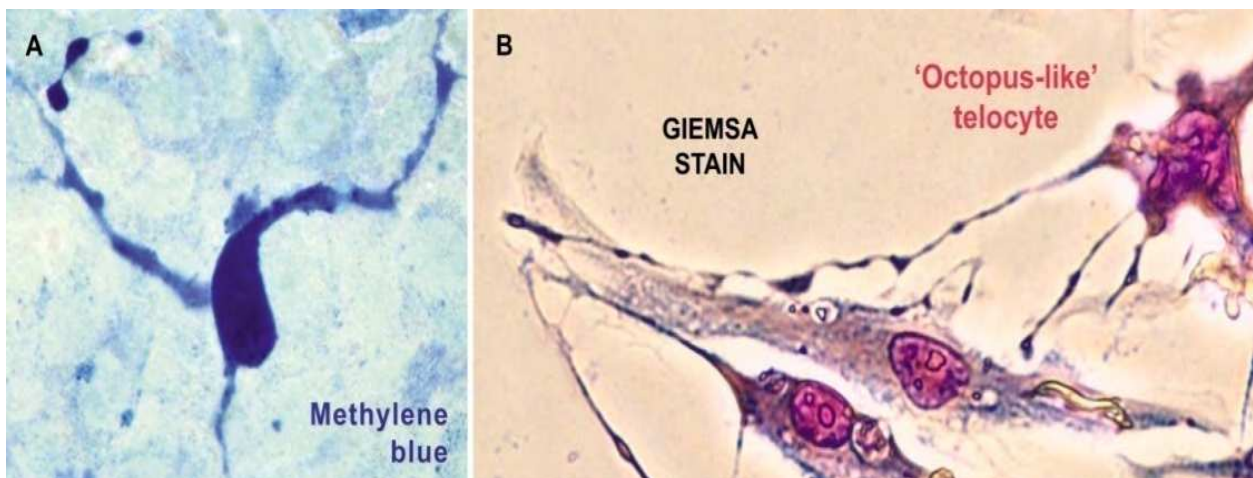


Fig. 8. A. Human pregnant myometrium. Primary confluent cultures (day 8) showing a telocyte with at least seven 'beads' per process. B. Human fallopian tube, preconfluent primary cell cultures. Conventional light microscopy, Giemsa staining. Original magnification 40x (A), 100x, oil immersion (B). Reproduced, with permission, from Ciontea et al., 2005 and Popescu et al., 2005a.

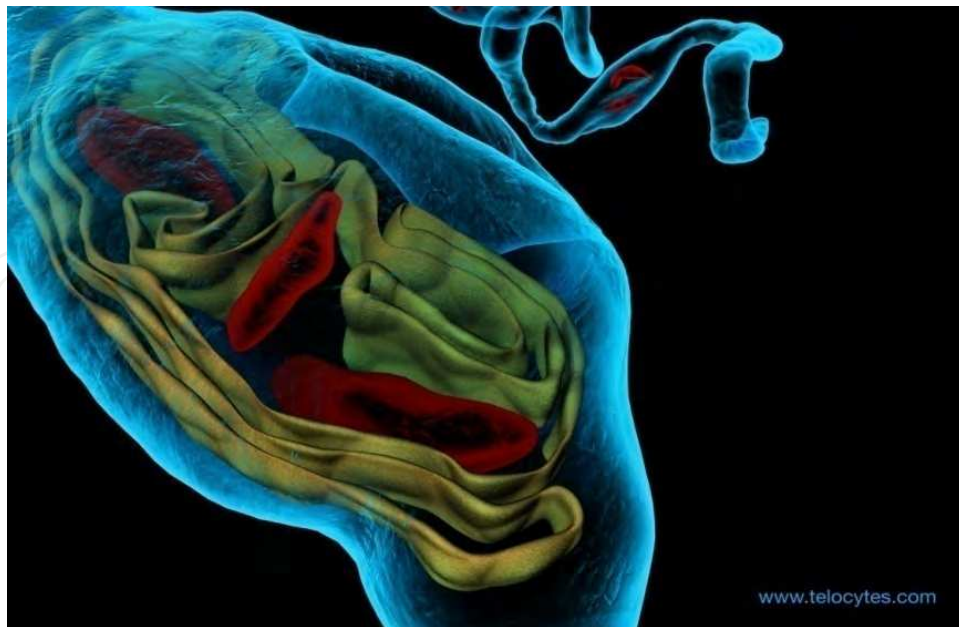


Fig. 9. The schematic drawing of a podom (blue), the dilated portion of a telopode. Note the podomic endoplasmic reticulum in yellow and the mitochondria in red.

- **Branching**, with a dichotomous pattern (Figure 10);

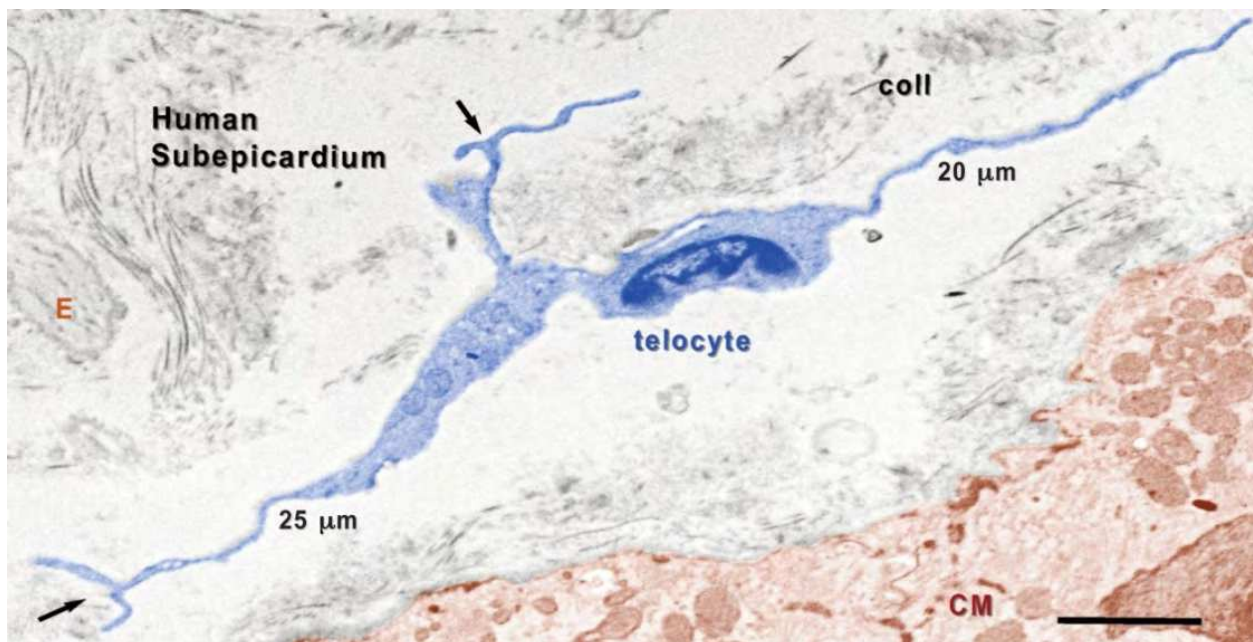


Fig. 10. Digitally coloured TEM image shows TC (blue) in human subepicardium, bordering the peripheral cardiomyocytes (CM, highlighted in brown). The TC has three telopodes, illustrating: a) the distinctive dichotomous pattern of branching (arrows); b) Tp are very thin at the emergence from the cell body; c) alternating podoms and podomeres. Note that some portions of podomeres have the same thickness as collagen fibrils, which makes observation under light microscopy impossible. E – elastin. Scale bar - 2 µm. Reproduced, with permission, from Popescu et al., 2010b.



- Organization in a labyrinthine system, forming a 3D network anchored by hetero- and homocellular junctions.

The concept of TC was soon embraced by other laboratories as well (Bani et al., 2010; Cantarero et al., 2011; Carmona et al., 2011; Eyden et al., 2010; Kostin, 2010; Zhou et al., 2010).

### 3. Sex steroids and TCs

The “sex hormones” – estrogens, progesterone, and androgens – are a special category of steroids. Their actions are mediated by intracellular receptors, generally known as nuclear receptors, acting as ligand regulating transcription factors (slow genomic mechanisms) as well as by membrane-associated receptors and signaling cascades (fast nongenomic mechanism) (Giretti & Simoncini, 2008; Tetel et al., 2009). Sex steroids are involved in the regulation of many functions in human organism, including reproduction and behaviour. Female genital organs, especially those directly involved in ovum fertilization and embryo implantation - fallopian tubes and uterus - are highly influenced by sex steroids.

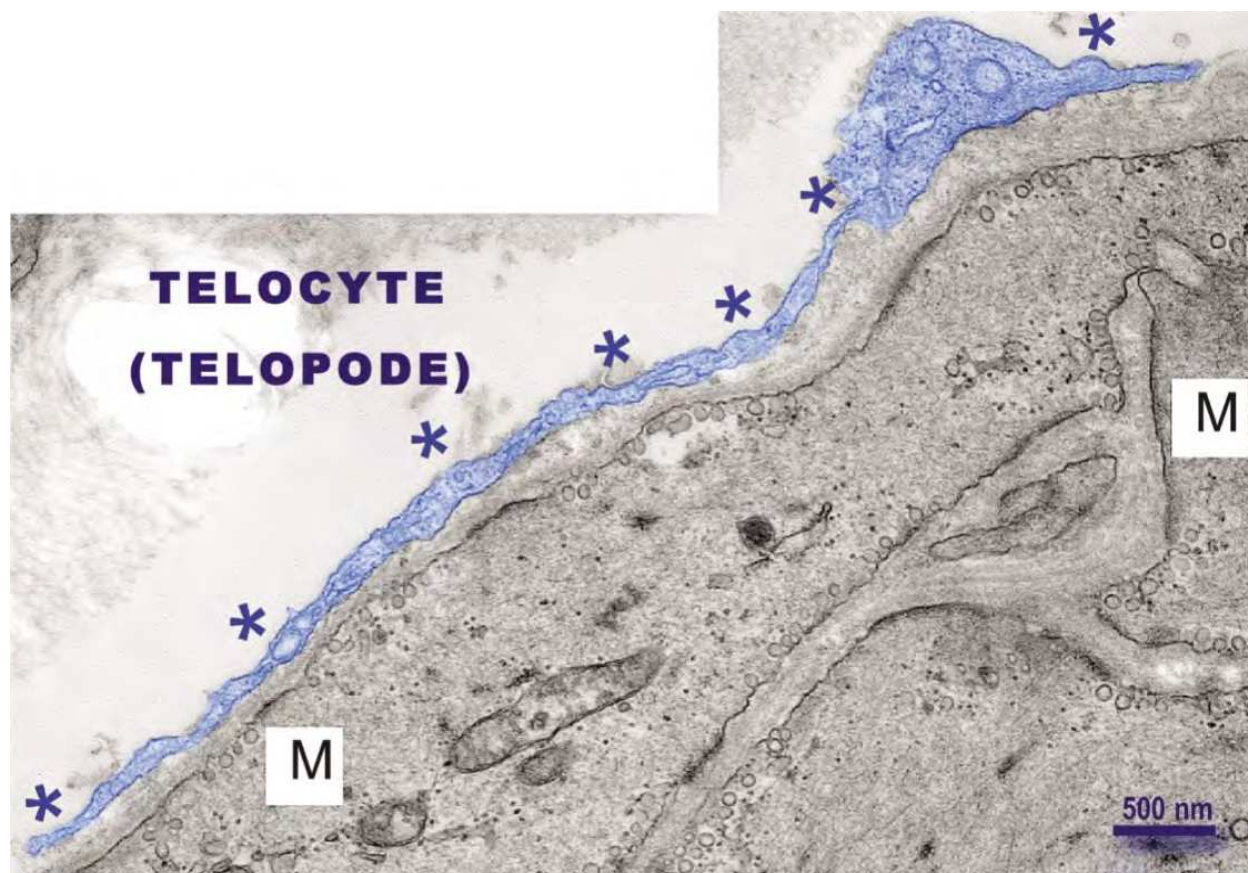


Fig. 11. Electron microscopy of non-pregnant human uterus. Note the telocyte covering smooth muscle cells (M). The telopode is digitally coloured in blue, marked with asterisks. Image obtained in 2006. Courtesy of Prof. M. Taggart (Newcastle University, UK) and Dr. Carolyn J.P. Jones (Manchester University, UK).

Connective tissue filling the space between epithelial, muscular and nerve tissue, found in the walls of these organs, abound in different cells. They are generally referred to as stromal



cells and are subclassified into fibroblasts, fibrocytes, myofibroblasts, interstitial cells, and mesenchymal cells. In recent years we describe a novel cell type – TCs – with a completely different silhouette. TCs are unequivocally recognized under transmission electron microscope on the basis of their most peculiar feature: TCs have extremely long prolongations, with a very thin and moniliform aspect (Figure 11).

Our laboratory was the first to describe the presence of sex steroid hormone receptors in TCs (D. Cretoiu et al., 2006, S.M. Cretoiu et al., 2009). TCs for cell cultures were obtained from the muscle coat of the fallopian tube and uterus, and analyzed by immunohistochemistry using monoclonal antibodies to determine the presence of estrogen receptor alpha (ER $\alpha$ ) and progesterone receptor (PR). TCs were enriched in primary culture by magnetically-activated cell sorting. The magnetic beads conjugated with goat anti-mouse IgG were incubated with monoclonal anti human CD117, considered to be specific for TCs. The cell suspension was then incubated with the magnetic beads and the supernatant was collected as the negative fraction. Culture medium was added to collect the remaining cells and the tube was removed from the magnet. This was considered as the positive fraction. We obtained  $1.8 \times 10^5$  cells in the positive fraction and  $4.8 \times 10^6$  cells in the negative fraction. After 9 days, cells grown on coverslips, in primary culture underwent subsequent examination.

### 3.1 TCs as steroid hormone sensors in human myometrium

TCs have been described in human uterine tissue under different names since 2004 : c-kit-positive cells (Shafik et al., 2004), m-CLIC (Ciontea et al., 2005), Vimentin-positive, c-kit-negative interstitial cells (Duquette et al., 2005), ICLC (Popescu et al., 2007; Hutchings et al., 2009). Our group found that myometrial TCs possessed very long cytoplasmic processes which, by *in vitro* Janus green B staining, were shown to contain numerous mitochondria. (Ciontea et al., 2005). TCs represented approximately 7% of the total cell number on random semi-thin myometrial tissue sections (Figure 12) stained with toluidine blue (Popescu et al., 2006).

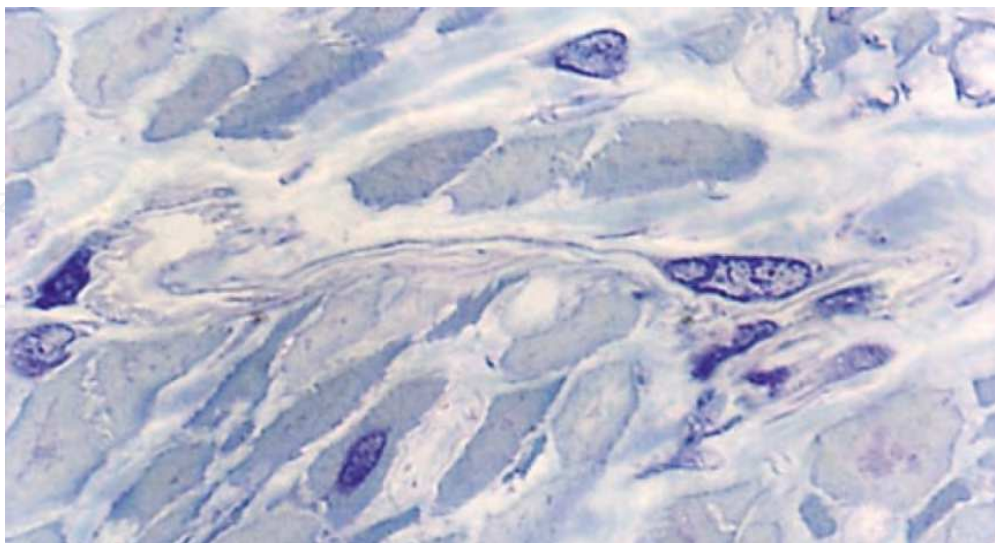


Fig. 12. Pregnant human myometrium (39 weeks of gestation). Semi-thin sections (0.5 - 1  $\mu$ m thick) of uterine muscular layer embedded in Epon resin and stained with toluidine blue. Note the very long process of the TC squeezing between obliquely cut smooth muscle cells. Original magnification 100x. Reproduced, with permission, from Hutchings *et al.*, 2009.

Uterine TCs display distinct features which avoid possible confusion with other types of interstitial cells. Methylene blue staining and Golgi impregnation, which was used for the first time by Cajal in 1892 (for ICC identification) are also necessary for identification of TC presence at tissue level or in cell culture (Figures 13, 14).

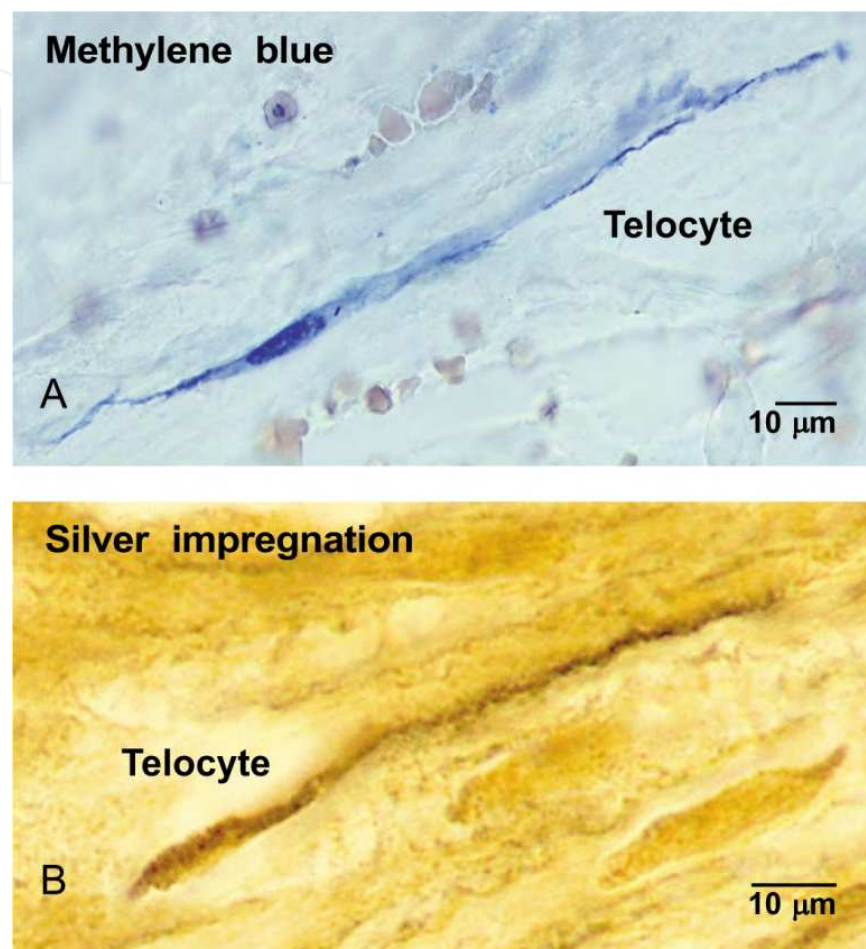


Fig. 13. Human myometrium. A. Methylene blue vital staining, before cryofixation (cryosectioning). Note the selective affinity of a telocyte for the blue dye. B. Silver impregnation after fixation and paraffin embedding. A pyriform telocyte with a very long, moniliform process. Original magnification: 1000x. Reprinted from European Journal of Pharmacology, 546, L. M. Popescu, C. Vidulescu, A. Curici, L. Caravia, A. A. Simionescu, S. M. Ciontea, S. Simion, Imatinib inhibits spontaneous rhythmic contractions of human uterus and intestine, 177-181, Copyright (2006), with permission from Elsevier..

At the myometrial level, TCs establish, through their TPs, vicinity relationships with capillaries and nerve fibers, as well as specialized contacts with other interstitial cells (e.g. macrophages, mast cells, lymphocytes, eosinophils) (Popescu et al., 2005b) (Figure 15). TCs interconnect with each other and with smooth muscle cells (SMC) through cell-to-cell point contacts or gap junctions. Interestingly, we found in uterine myocytes typical 'Ca<sup>2+</sup> release units (caveolae, sarcoplasmic reticulum and mitochondria) in the vicinity of gap junctions (Figure 16).



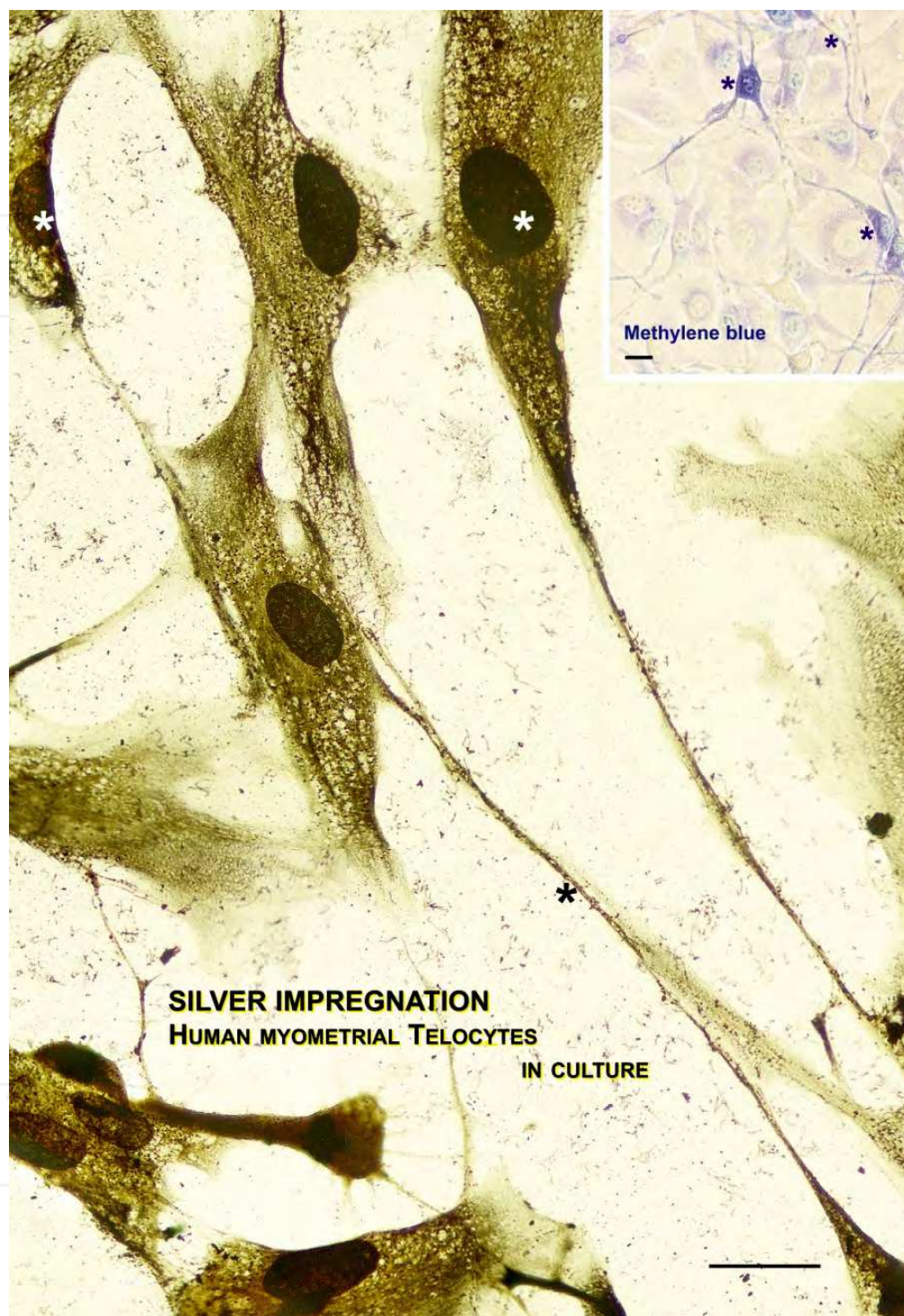


Fig. 14. Photographic reconstruction of human TCs in culture establishing contact with smooth muscle cells. From our experience, silver impregnation is one of the choice methods for revealing the typical moniliform aspect of TCs in culture. Inset- the same interlaced distribution of TCs (\*) using methylene blue vital staining. Both methods reveal weaker myocyte staining. Scale bar 10  $\mu$ m. Reproduced, with permission, from Cretoiu et al., 2006.

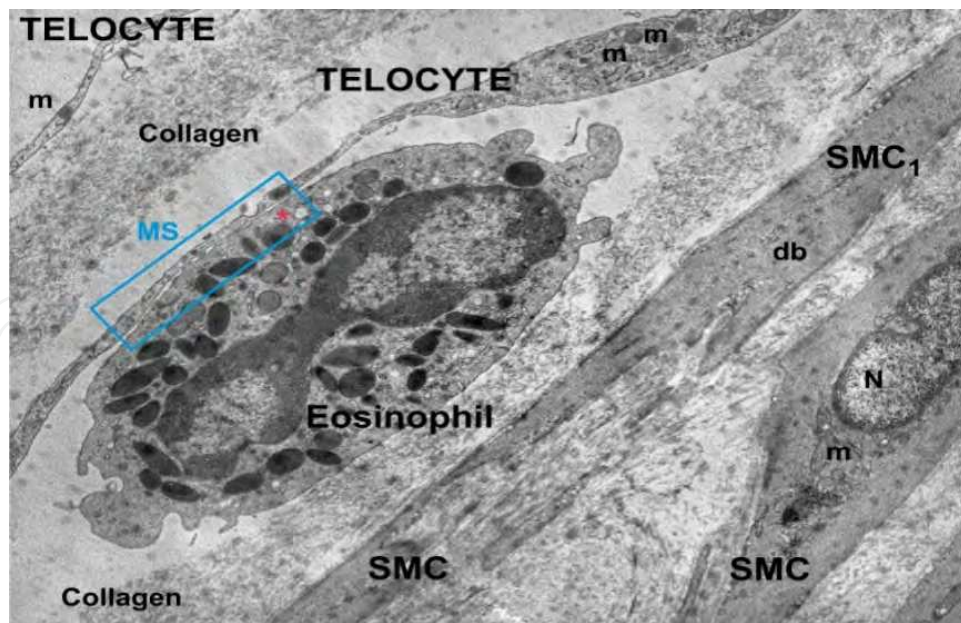


Fig. 15. Rat myometrium: TEM; original magnification 7100x. A multi-contact synapse (MS) between a telocyte and an eosinophil, in the neighbourhood of smooth muscle cells (SMC); m, mitochondria; N, nucleus; db, dense bodies; Note presence of mitochondria (\*) in the synaptic vicinity, typical of chemical synapses. Reproduced, with permission, from Popescu et al., 2005b.

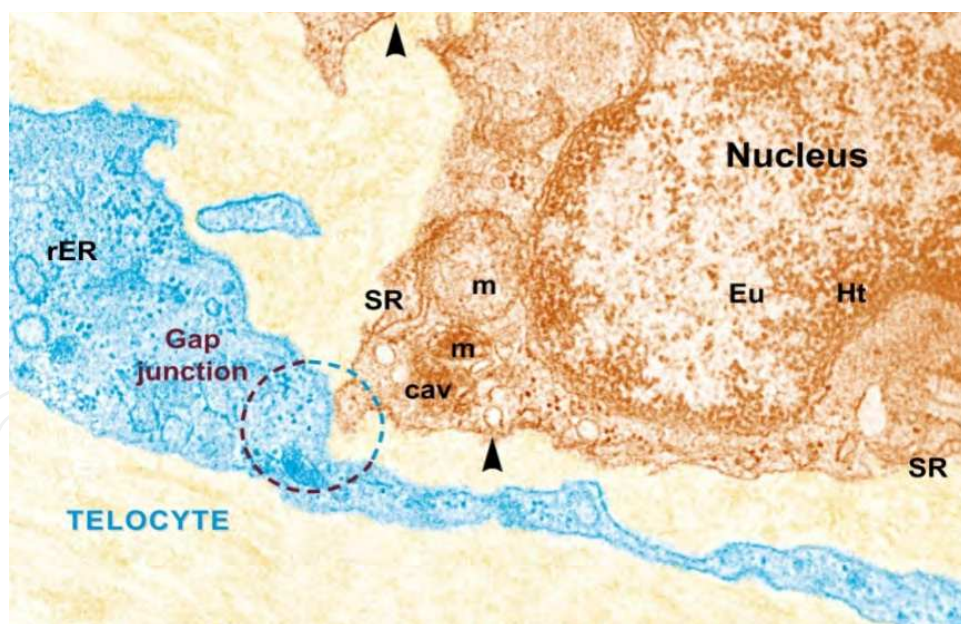


Fig. 16. Digitally-coloured TEM image of a TC in rat myometrium: TC (blue), smooth muscle cells (Sienna brown). Note the 'Ca<sup>2+</sup>-release units' (caveolae, sarcoplasmic reticulum and mitochondria) in the cytoplasmic region where smooth muscle sarcolemma comes into close contact with TC plasmalemma. Original magnification: x15000. SMC = smooth muscle cells; Ht = heterochromatin; Eu = euchromatin; rER = rough endoplasmic reticulum; SR = sarcoplasmic reticulum; m = mitochondria; cav = caveolae (arrowheads). Reproduced from Ciontea et al., 2005.



Currently, there is no established panel of antibodies for TC immunophenotyping. We previously reported that antibodies against CD117/c-kit result in weak and sometimes inconsistent TC immunostaining. Most of CD117 positive cells co-express CD34 and vimentin (Ciontea et al., 2005, Popescu et al., 2006) (Figure 17).

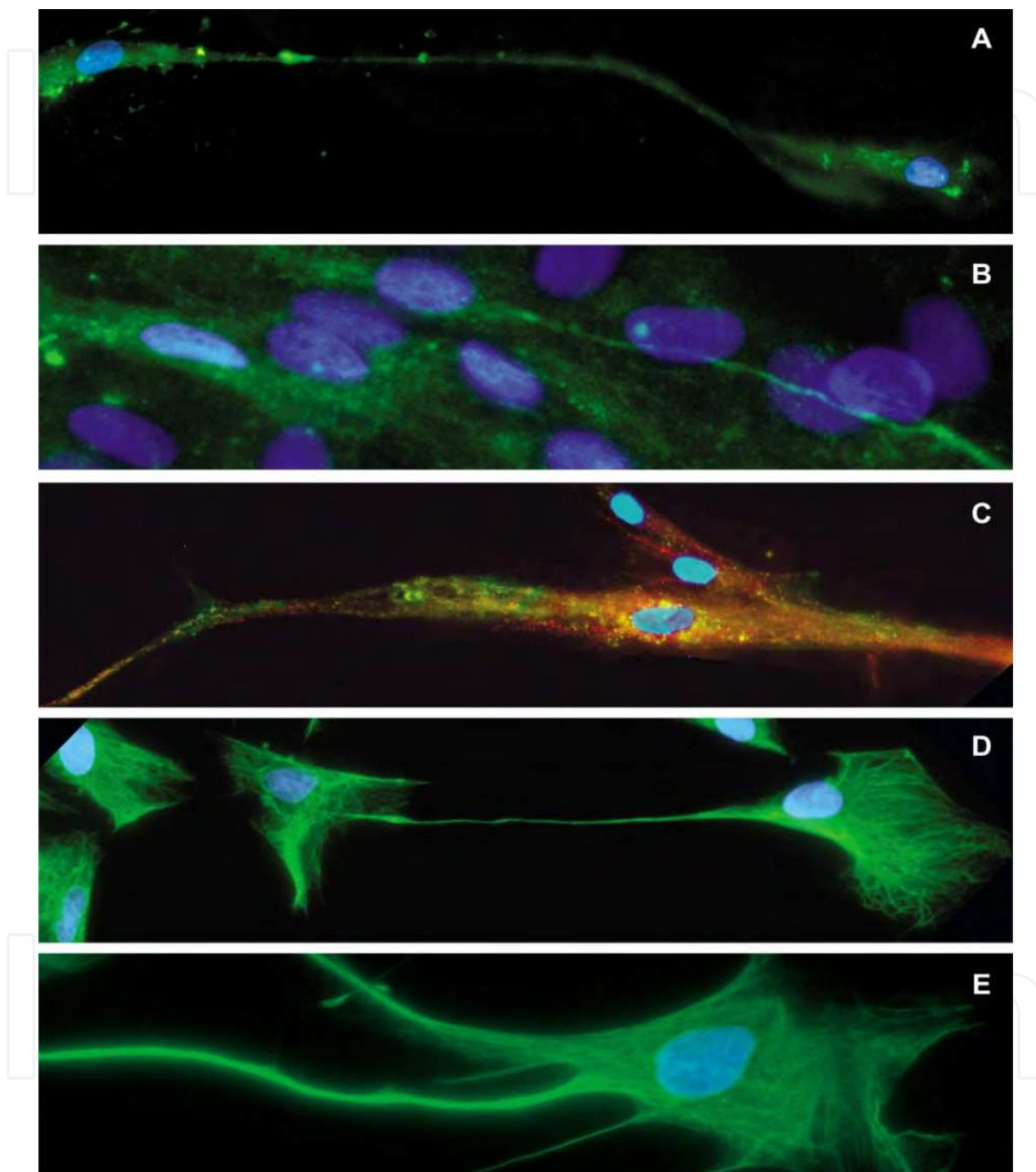


Fig. 17. Human myometrium cells in culture (the 2nd passage): c-kit (green in A, B), c-kit and CD34 (red and green, respectively, in C) and vimentin (green in D, E). Cells which display the morphologic TC feature (long, moniliform processes) express c-kit and contact adjacent cells (A-C). Some cells suggestive of TCs co-express c-kit and CD34 (C). The characteristic cell processes are immunoreactive for vimentin and establish connections with nearby cells (D and E). Original magnification 60x, nuclear counterstaining with Hoechst 33342 (blue). Reproduced, with permission, from Ciontea et al., 2005.

However, using immunohistochemistry alone, we cannot differentiate between interstitial cells since. For instance, c-kit positive cells could be stem cells, mast cells (Terada, 2009; Cinel et al., 2009), or TCs. TCs have a thin rim of cytoplasm. Their TPs (often up to 100 nm thick) can be undetectable under an immunofluorescence microscope, falling below light microscopy resolution. Electron microscopy is fundamental in identifying TCs with their peculiar appearance, having extremely long and moniliform processes with a dichotomous branching pattern which sometimes gave them a dendritic aspect. Table 1 distinctively draws a demarcation line between TCs, canonical ICC and fibroblasts.

Once TCs were established as cellular components of the hormonally responsive uterine tissue, we addressed whether they might express steroid receptors. Because, *in situ*, it may be difficult to observe morphological differences between the tightly packed myometrial cells and TCs, we chose to dissociate myometrial tissue to determine which cell type(s) expresses ER- $\alpha$  or PR-A. By Immunocytochemistry, we identified two cell types on the basis of their ER and PR immunoreactivity: a. TCs, which showed intense nuclear and weak cytoplasmic immunostaining for both ER and PR, (Figure 18 A, B); b. myocytes and/or fibroblasts which remained relatively unstained (Figure 18 A). Using double immunostaining for CD117/c-kit, we confirmed that cells which stained positive for ER and PR were TCs, (Figure 18 C, D). Double immunofluorescence confirmed the same distribution of PR and ER in c-kit positive cells, intense at nuclear level and weak in the cytoplasm (Figure 19 A-H).

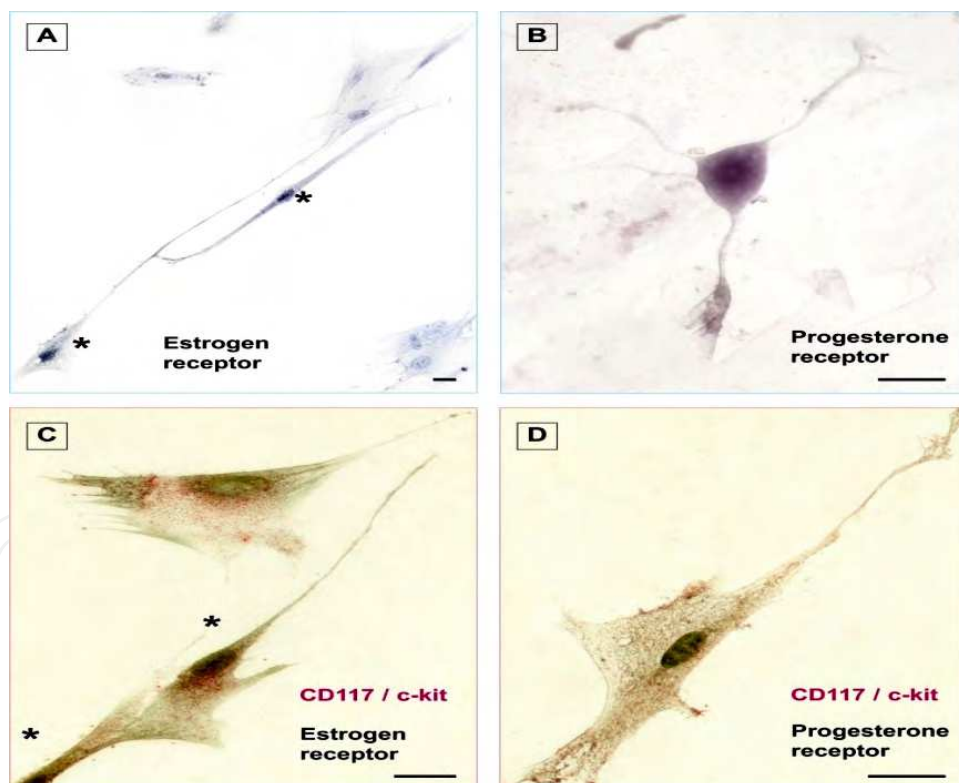


Fig. 18. A-D. Human myometrial cell culture, fourth passage. Immunocytochemical staining for estrogen and progesterone receptors. A. Immunocytochemical detection of estrogen receptor - dark stained nuclei (\*), counterstaining with methyl green for negative nuclei. B. TCs stained positive for progesterone receptor. C. Doublestaining (\*) for CD117/c-kit (red) and estrogen receptor (black). D. Double staining for CD117/c-kit (red) and progesterone receptor (black). Scale bar = 10  $\mu$ m. Reproduced, with permission, from Cretoiu et al., 2006.



		Interstitial cells of Cajal (ICC)	Myometrial telocytes	Fallopian tube telocytes	Fibroblasts
Cell shape		Oval or spindle-shaped body	Spindle or stellate body		Polymorphic body
Nucleus		Oval, mostly euchromatic	Oval, heterochromatic under nuclear membrane		Oval, euchromatic with 1-2 visible nucleoli
Cytoplasm	Smooth ER	++	+	+	+/-
	Rough ER	+	+	+	+++
	Golgi apparatus	+	+		+++
	Mitochondria	+++	++	+	+
	Intermediate filaments	++	+	+	+
	Microtubules	+	+	+	+
	Thin filaments	+	+	+	+
	Calcium releasing units	n.a.	present	n.a.	n.a.
Other structures	Caveolae	+	+	++	-
	Basal lamina	0	+/-	+/-	-
Immunohistochemical markers		c-kit	Co-localization of c-kit, CD34 and connexin 43, lack of prolyl 4-hydroxylase		Prolyl 4-hydroxylase
Intercellular contacts	Nerve endings	++	+	+	-
	Blood vessels	n.a.	+		-
	Immune cells	n.a.	+++		-
	Smooth muscle cells	+	+	+	-
	Other interstitial cells	+	+	+	-
	Gap junctions	+	+		-

Table 1. Morphological aspects, semi-quantitative data concerning the ultrastructural elements (transmission electron microscopy) and specific markers of telocytes compared to archetypal enteric interstitial cells of Cajal (ICC) and fibroblasts. Adapted from Hutchings et al., 2009.

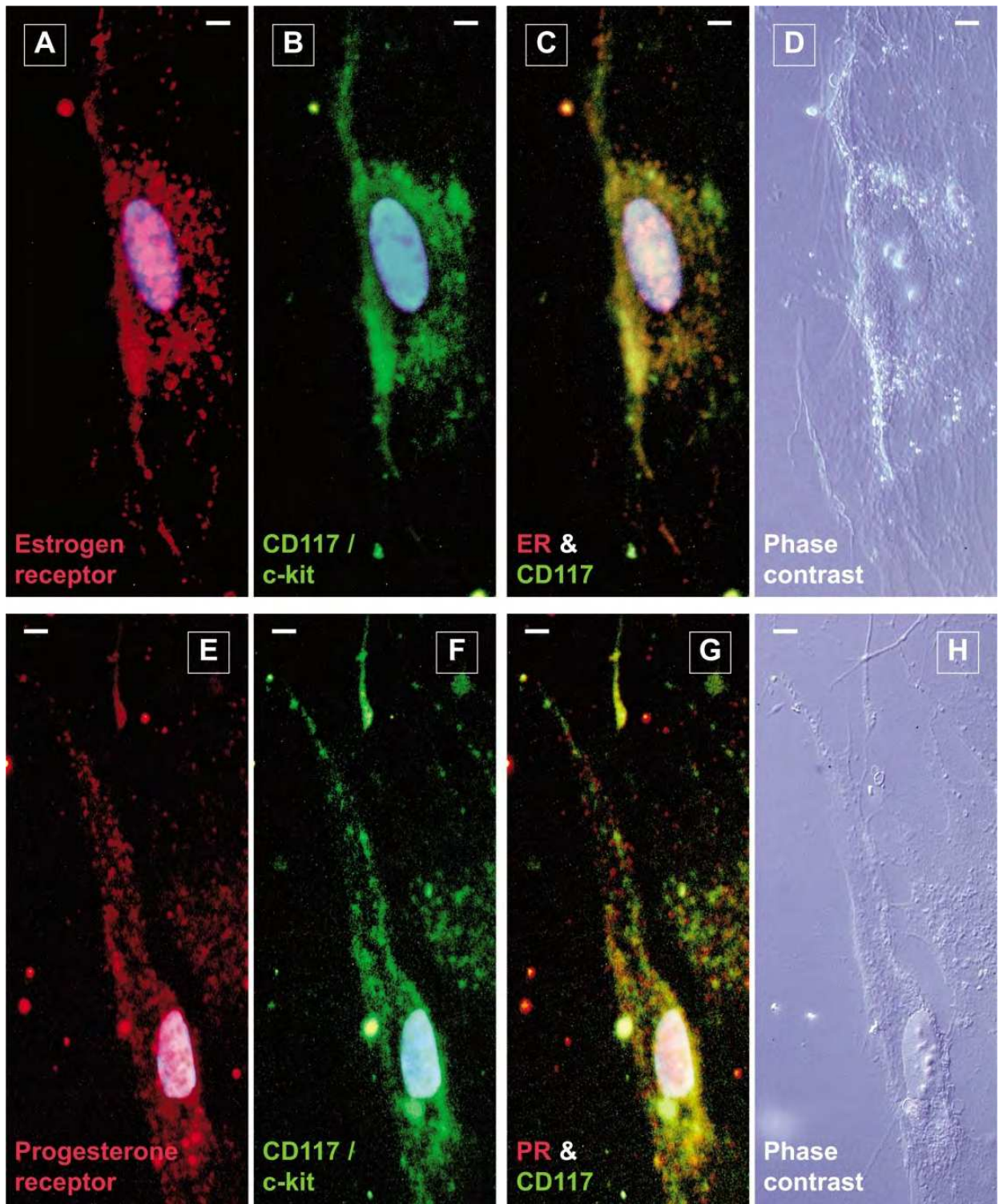


Fig. 19. A-H. Human myometrial cell culture, fourth passage. Immunofluorescent labeling for estrogen (A) and progesterone (E) receptor (red) which appear both inside the nucleus and in cytoplasm. c-kit/CD117 only (green) found in the cytoplasm (B, F) and double labeling for both markers (C, G), where co-expression appears as yellow areas. Hoechst 33342 (blue) for nuclear counterstaining. Phase contrast microscopy focused on the same cells, typical TCs with long, moniliform prolongations (D, H). Scale bar = 2  $\mu$ m. Reproduced, with permission, from Cretoiu et al., 2006



3.2 TCs of human fallopian tube express ER and PR

Fallopian tubes are very important in human reproductive medicine playing active roles in such as gamete transport and final maturation, capacitation of sperm, ovum fertilization, early embryo development and delivery of embryo to the uterus. Each anatomic region (infundibulum with fimbria, ampulla, isthmus and intramural segment) seem to perform specific functions. Our discovery of novel interstitial cells in Fallopian tube tissue in 2005 (Popescu, 2005), which we now know to be TCs, has brought more attention to studies of this tissue. TCs are resident (dominantly) in fallopian tube lamina propria and in between smooth muscular fibres. The TCs percentage in the fallopian tube wall discloses the following areas of interest, starting from the basement membrane toward the serosa: area in the lamina propria found in close vicinity of the basement membrane (18±2%); area containing the entire lamina propria thickness (~8%); muscularis per se (7.8±1.2%) and the remaining zone beneath serosa (was not assessed). We concluded that the TC spatial distribution gradient decreases from the sub-epithelial area to the serosa. In lamina propria the percentage of TCs represent on average 11.0±0.6% of all cells. TC cellular bodies can take on various shapes: pyriform (having only one prolongation), (50%); spindle (with two opposite prolongations), (30%), triangular (15%) and other shapes with more than three prolongations (5%). Tubal TC immunophenotyping was performed by correlating morphology with immunohistochemistry using a panel of 15 antibodies (Table 2).

Antibody	Clone	Dilution	Source	IHC positivity
CD117/c-kit	polyclonal	1:100	DAKO	+ + + +
CD34	QBEnd10	1:100	Biogenex	+ + +
S-100	polyclonal	1:500	DAKO	+ +
α-SMA	1A4	1:1500	Sigma	+
CD57	NK1	1:50	DAKO	+
nestin	5326	1:100	Santa Cruz	+
desmin	D33	1:50	DAKO	-
vimentin	V9	1:50	DAKO	+
NSE	BBS/NC/VI-H14	1:50	DAKO	+
GFAP	6F2	1:50	DAKO-	
CD68	PG-M1	1:50	DAKO	+
CD62P	1E3	1:25	DAKO	-
CD1a	CD1a-235	1:30	Novocastra	-
Chromo A	LK2H10	1:50	Novocastra	-
PGP9.5	10A1	1:40	Novocastra	-

Table 2. Summary of immunohistochemical results for telocytes from human fallopian tube. The intensity of telocyte reactivity was assessed semi-quantitatively using an adaptation of the Quick score method. Intensity: Negative (no staining of any cellular part at high magnification):-; Occasionally weak positive: +; Low (only visible at high magnification):+; Medium (readily visible at low magnification):+ +; High (strikingly positive at high magnification):+ + +; Strong (strikingly positive even at low magnification):+ + + +.

TCs with characteristic morphology (one or more very long, thin processes, sometimes with 'beads-on-a-string' appearance, that arise from pyriform, stellate or spindle shaped cell bodies) were found to express c-kit. Some of the TCs co-express CD34, desmin, vimentin and even  $\alpha$ -SMA. TCs in the fallopian tube fulfill the ultrastructural identification criteria and are definitely distinct from fibroblasts (Figure 20).

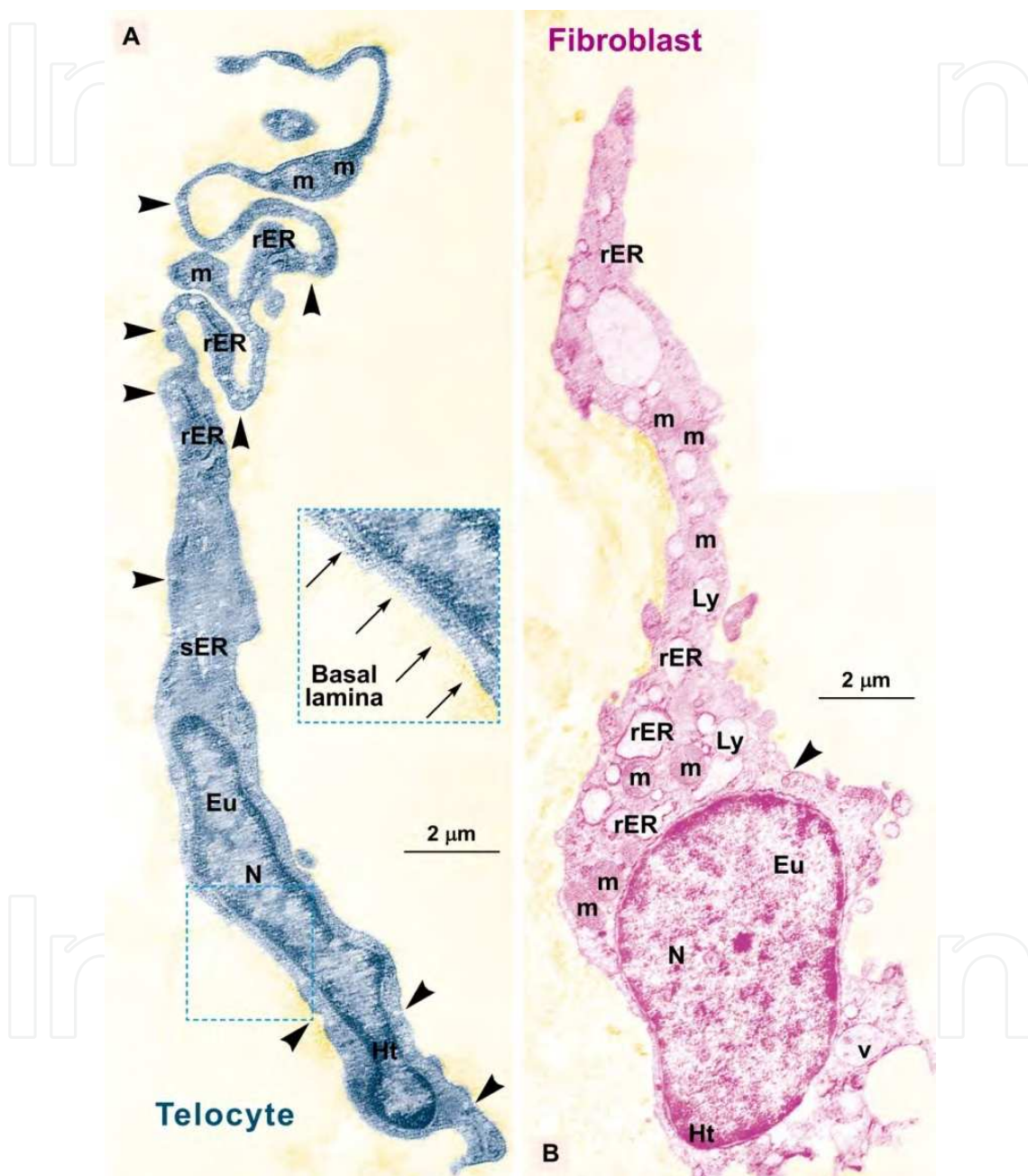


Fig. 20. (A) A telocyte compared to (B) a fibroblast from the same TEM ultrathin section of human fallopian tube (digitally coloured images). N = nucleus; Eu = euchromatin; Ht = heterochromatin; rER = rough endoplasmic reticulum; sER = smooth endoplasmic reticulum; m = mitochondria; v = vacuolae; Ly = lysosomes; arrowheads indicate caveolae. At least 29 caveolae can be counted in the TC's convoluted process (A, upper part). Reproduced, with permission, from Popescu et al., 2005a.

TCs were enriched in primary culture by magnetically-activated cell sorting, after being identified as c-kit positive cells with characteristic morphology. Indeed TCs were found in a higher percentage after magnetic cell sorting: approximately  $30 \pm 0.8\%$  ( $n = 516$ ) compared to  $9.9 \pm 0.9\%$  ( $n = 324$ ) (Popescu, 2005). The sorted populations underwent subsequent passages, because according to our previous experience, the number of TCs increases with each passage.

*In vitro* double staining on Fallopian tube samples showed that desmin-positive cells (SMC) tested negative for ER- $\alpha$  or PR-A and c-kit-positive cells (telocytes) tested positive for ER- $\alpha$  or PR-A (Fig. 21 A,B). Moreover, double staining for c-kit (green fluorescence) and PR-A or ER- $\alpha$  (red fluorescence) revealed that only cells positive for c-kit were also positive for ER- $\alpha$  and PR-A at nuclear level (Fig. 22 A-F). PR-A expression at nuclear level was more intense than for ER- $\alpha$ . SMC were weakly positive or completely negative.

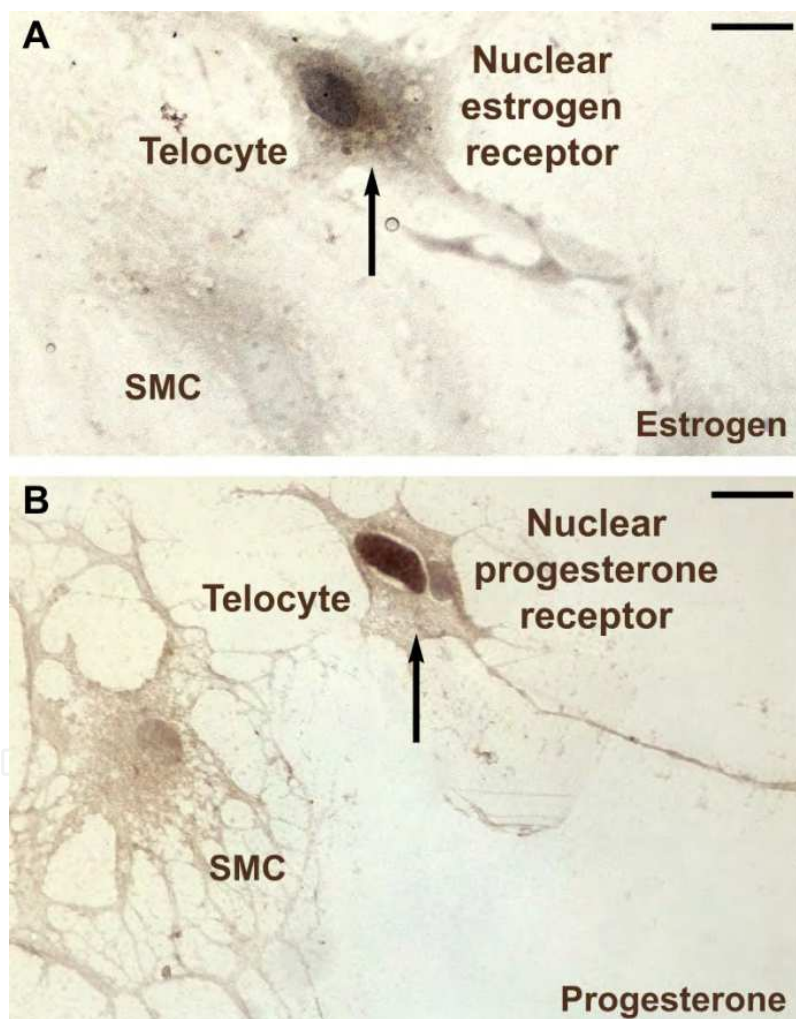


Fig. 21. A,B. Human Fallopian tube cell culture, fourth passage. The expression of ER- $\alpha$  (A) and PR-A (B) demonstrated by immunocytochemical staining. TCs (arrows) stained positive for ER and PR (brown nuclei). Scale bar = 5  $\mu$ m. With kind permission from Springer Science+Business Media: Journal of Molecular Histology, Interstitial Cajal-like cells of human Fallopian tube express estrogen and progesterone receptors, 40, 2009, 387-394, Cretoiu, S.M.;Cretoiu, D.;Suciu, L.&Popescu, L.M., figure 4.



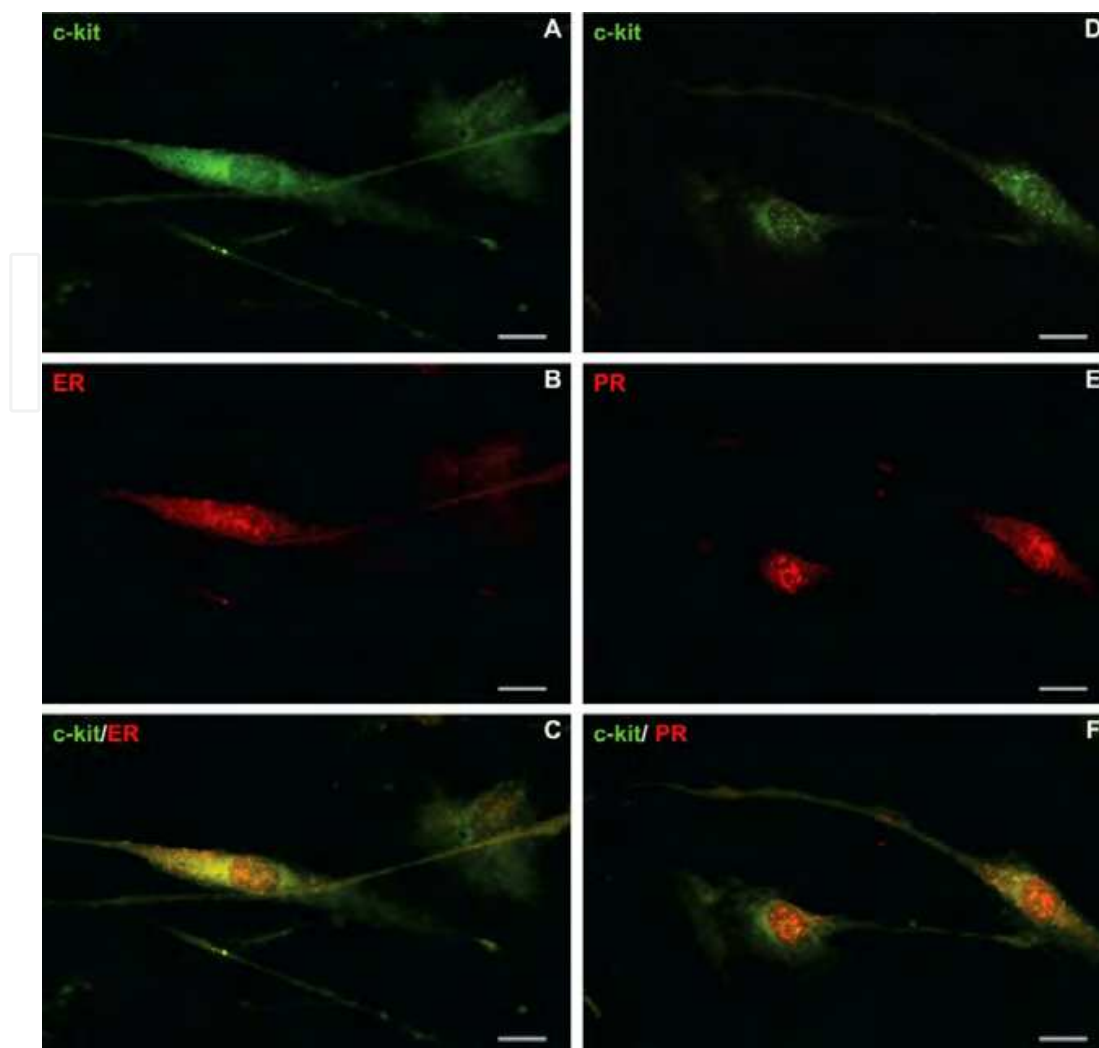


Fig. 22. A-F. Human Fallopian tube cell culture, sixth passage. Immunofluorescent labeling for c-kit/FITC (A) and ER- $\alpha$ /Alexa Fluor 546 (B), and superimposed images to show colocalization (C). c-kit fluorescence of TC (D) and PR-A fluorescence (E). Superimposed labeling for both markers (F), where c-kit (green) is localized only in the cytoplasm and PR-A is expressed in the TC nuclei (red). Scale bar = 5  $\mu$ m. With kind permission from Springer Science+Business Media: Journal of Molecular Histology, Interstitial Cajal-like cells of human Fallopian tube express estrogen and progesterone receptors, 40, 2009, 387-394, Cretoiu, S.M.; Cretoiu, D.; Suciu, L. & Popescu, L.M., figure 5.

## 4. Possible TC roles

### 4.1 TCs and signaling processes

Recently, some of the TCs located on the extracellular matrix of blood vessels were described as having a primary cilium (Cantarero et al., 2011). The presumed functions of such a non-motile cilium could be:- organizer of the mitotic spindle (Alieva et al., 2004), sensory organelle involved in signal transduction - hedgehog pathway (Singla et al., 2006), mechanical sensing and mechano-chemical conversion in endothelial cells (Egorova et al., 2011; Nauli et al., 2008). By analogy, we can presume that TCs could be involved in the signaling process if located near the stromal colony-forming cells/units in human endometrium or might act as stretch sensors if located near smooth muscle structures in both Fallopian tubes and uterus.

TPs usually form and release vesicles (or exosomes) which might indicate the possible involvement of TCs in intercellular communication. For example, in the heart, heterocellular communication between TCs and cardiomyocytes seems to occur by shed vesicles and close apposition (Gherghiceanu et al., 2011). Intercellular signaling can occur by two mechanisms: a paracrine and/or juxtacrine secretion of small signaling molecules and shedding microvesicles which transport 'horizontal' "packets" of macromolecules to the target cells, modifying their physiology. These vesicles can even transport DNA or RNA among neighbouring cells, inducing epigenetic changes (Akao et al., 2010; Zomer et al., 2010). We suspect a complex interplay between TCs, immune cells, cells involved in epithelial or even myometrial regeneration and cancer spreading (Pap et al., 2011).

#### 4.2 TCs and stem cells

It is known that the remodeling events which take place in the uterus (endo- and myometrium) during implantation and pregnancy are coordinated by sequential actions of estrogen and progesterone (Szotek et al., 2007). TCs, as a special type of stromal cells, could be involved in uterine remodeling since they express ER and PR, and are also located in the lamina propria, beneath the epithelium and in between myocytes. There are fundamental studies that provide evidence that both epithelial and stromal stem/progenitor cells are found in human and mouse uterus (Gargett et al., 2008). The discovery of relationships between TCs and these uterine stem cells could provide new insights into the pathophysiology of various gynecological and obstetrical disorders. In 2009, Shynlova et al. proposed a new model of phenotypic modulation of uterine myocytes during pregnancy. These changes evolve in an early proliferative phase, an intermediate phase of cellular hypertrophy and matrix elaboration, a third phase in which the cells assume a contractile phenotype and the final phase in which cells become highly active and committed to labour. The final phase of myometrial differentiation is postpartum uterine involution. These stages are in fact the result of integration of endocrine signals and mechanical stimulation of the uterus by the growing fetus (Shynlova et al., 2009). In our opinion, TCs could be themselves stem cells (Popescu et al., 2011b), playing a part in muscle regeneration (Popescu et al., 2011a), these processes possibly depending on steroid hormones receptors.

#### 4.3 TCs and immune cells

TCs often establish contacts with targets, such as smooth muscle cells, nerve fibres, and capillaries (Popescu et al., 2011). Over time, we also described close contact between TCs and cells of the immune system, found in the interstitial space (e.g. eosinophils, plasma cell, etc.). We considered that this is a new type of synapse - the stromal synapse - in addition to the existing neuronal and immunological synapse (Popescu et al., 2005b). The intercellular contact can either be "plain" uniform or "kiss-and-run" multicontact, based on synaptic cleft tracing.

### 5. Perspectives

TCs can be putative cellular mechanotransducers in smooth muscle tissue. They may sense and translate stretch information for the nucleus, and activate genes responsible for protein synthesis which can influence the surrounding cells by juxta- or paracrine mechanisms. TCs could also be 'hormonal sensors' in human myometrium and the Fallopian tube since they express estrogen and progesterone receptors *in vitro*. The presence of steroid hormone receptors suggests that TCs could also be responsible for myogenic contractility modulation under hormonal control, either by transferring bioactive molecules (towards cells from an

endometrial stem cell colony), either by direct stimulation of target cells (immunoreactive cells). Recent evidence suggests that TCs may play a role as putative actors in neo-angiogenesis (Manole et al., 2011).

## 6. Conclusions

In conclusion, the presence of steroid hormone receptors suggests that TCs could behave as sensors controlling the Fallopian tube peristalsis by signaling mechanisms (para- or juxtacrine), depending on ovarian hormone levels, by opposite effects (accelerated by estrogens and delayed by progesterone). Our findings might even explain infertility in patients without any proven Fallopian tube abnormalities. At uterine level, the discovery of TCs is fundamental for a totally new approach regarding the mechanisms controlling myometrial contractility during and outside pregnancy. The evidence for steroid hormone receptors at the level of myometrial TCs might open a path towards the understanding of contractility modulation using steroid hormones. This effect could be the result of intercellular connections between TCs and myocytes. The particular structure of the podoms with energetic (mitochondria) and functional (proteins from ER) resources favours the extension of Tp in the extracellular environment for signalling purposes of for intercellular communication. The steroid receptors occurrence in TCs could also suggest that these cells participate in the exchange of genetic information with other cells (myocytes, immune cells, nerve fibres) or for sensing changes in stromal microenvironment. If some of the supposed functions will be proven, TCs could be used in the future as molecular tools for delivering biological drugs at genital organs level.

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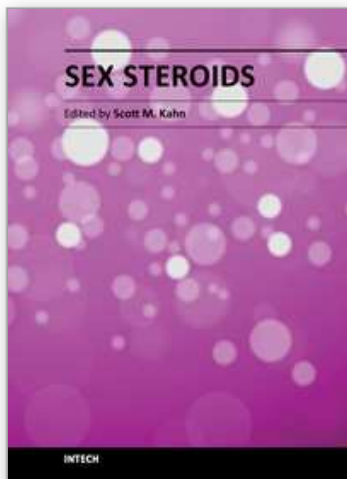


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## **Sex Steroids**

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This book, entitled "Sex Steroids", features a valuable collection of reviews and research articles written by experts in signal transduction, cellular biology, diseases and disorders. "Sex Steroids" is comprised of four sections, "The Biology of Sex Steroids", "Sex Steroids, Memory, and the Brain", "Sex Steroids and the Immune Response", and "Therapy"; individual chapters address a broad range of recognized and predicted functions and applications of sex steroids. "Sex Steroids" is intended to provide seasoned veterans as well as newcomers to this area of research with informative, resourceful, and provocative insights. Readers of "Sex Steroids" should emerge with an appreciation and understanding of the multitude and complexity of biologic processes attributed to these important hormones, and possible future directions of research in this fascinating and ever evolving field.

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