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# Human Myometrial Interstitial Cajal like Cell (Telocyte) in Preterm and Full Term Labour: Histological and Immunohistochemical Studies

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## ABSTRACT

**Introduction:** Preterm labour is a common obstetric problem. The human myometrium is formed of two types of cells namely smooth muscles and interstitial Cajal like cells or telocytes which express both estrogen and progesterone receptors**.**

**Aim of the work:** This study aimed to know how many telocytes are present in the pregnant uteri at time of labour in the different gestational ages and the possible role of telocytes in preterm labour.

**Patients and methods**: 10 pregnant women were included in the study planned to do cesarean section. They were divided into two groups: **Group I**: included 5 cases whose gestational age was less than 37 weeks (preterm). **Group II**: included 5 cases whose gestational age was more than 37 weeks (full term). Specimens were obtained from the myometrium at the time of the operation and processed for histological and immunohistochemical study.

**Results:** H&E stained sections revealed hypertrophied smooth muscle fibers with different orientations. Methylene blue stain revealed telocytes between the smooth muscle fibers as a branched cell with small cell body and thin long processes. CD 117 (ckit) immunostaining revealed an apparent increase in the number of telocytes in preterm cases (**Group I**) compared to full term cases (**Group II**). Morphometric study revealed a significant increase in the number of ckit positive telocytes in preterm cases compared to the full term one.

**Conclusion**: telocytes are present in the pregnant uteri at different gestational ages with a significant increase in their number in the preterm cases; they may have a possible role in preterm labour.

**Keywords:** Myometrim, telocytes, preterm, full term, c kit.

## INTRODUCTION

Preterm labour is a common obstetric problem associated with fetal and maternal complications. Many factors are involved in the regulation of uterine contractility, as mechanical stretch by the growing fetus, intercellular signaling changes (endocrine/paracrine signaling) or the presence of inflammatory cells during spontaneous normal labour [1-5]**.**

The human myometrium is consisted of two types of cells involved in its contractility: smooth muscle fibers and interstitial cells that are named telocytes (TCs) or interstitial Cajal like cells [6]**.** Cajal cells were firstly described in 1911 by the Spanish neuroanatomist Cajal in the wall of gastrointestinal tract. Cells resembling Cajal cells, but outside the gastrointestinal tract they are named interstitial Cajal like cells or (TCs) [7].

It was reported that telocytes are present in many organs in the body: heart, lung, pancreas, skeletal muscles, parotid gland and urinary bladder [8-13].

Regarding the female reproductive system, telocytes were found to be present in resting mammary gland stroma [14], human fallopian tubes, normal human vagina [15] and pregnant and non pregnant uteri [16&17].

Telocytes were found to have contact with each other and with the smooth muscle cells by gap junctions and also with the nerve bundles and blood vessels [7]. Furthermore, they express estrogen and progesterone receptors in non-pregnant uterus and during the fertile period [18].

The presence of steroid hormone receptors suggested that telocytes could be responsible for myogenic contractility modulation under hormonal control [16]. Most of the markers expressed by the interstitial Cajal like cells are in common c-kit, CD34 and Vimentin [19].

Regarding the function of telocytes, it is suggested to have different roles according to their location. **Horn *et al.*** suggested that



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telocytes could prevent premature uterine contraction in midgestational period [20]. Others reported its role in angiogenesis [6]. Furthermore, they act as mechanoreceptors in rat mesentry [21].

**Aim of work**

The present study was conducted to evaluate how many telocytes are present in pregnant myometrium at time of labour in preterm and full term cases and their possible role in preterm labour.

## PATIENTS AND METHODS

After the Research and Ethics Committee have approved the protocol of this study, it was carried on 10 pregnant women planned to have a lower segment cesarean delivery. Specimens were taken in the period from October 2013 to December 2013. Before the operation a brief explanation of the study was given to each woman then a written consent to participate in the study was obtained.

Women were classified in 2 groups;

**Group I**: included5 cases, those who were pregnant less than 37 weeks of gestation

**Group II**: included 5 cases, those who completed 37 weeks of gestation.

During the operation a slice of uterine musculature was taken from the edge of the wound.

***Methods***

Specimens were fixed in 10% formalin and processed for study with light microscopy by the following stains:

(1) Hematoxylin and eosin for general histological examination [22].

(2) Methylene blue for demonstration of telocytes[19].

(3) Immunohistochemistry: anti CD117(c-kit) for demonstration of telocytes[23&24].

**Immunohistochemistry:**

**Staining procedure**: formalin-fixed, paraffin-embedded tissue sections were done and mounted on coated glass slides. Sections were deparaffinized and rehydrated through descending grades of alcohols and then put in distilled water for 5 min. Endogenous peroxidase activity was blocked with 0.6% hydrogen peroxide for 10 minutes using peroxidase blocking reagent. Antigen retrieval was done by boiling slides in EDTA buffer solution (pH 8.0). The slides were microwaved at a high medium for 15 min. The sections were incubated with 1/100 CD117 at room temperature, washed and incubated with biotinylated secondary antibodies, and then with the avidin–biotin complex. Finally, sections were counter stained with hematoxylin, dehydrated, cleared and mounted. CD117positive cells appeared brown and nuclei appeared blue. A negative control section was done with omission of the 1$^{ry}$ antibody. Universal kits and primary antibody (CD117) were purchased from Thermo fisher scientific company.

**Morphometric and statistical analysis**

Using Leica Qwin 500 C microscope, the number of c-kit-positive telocytes per high-power field (HPF) was determined in the myometrium. C-kit positive telocytes were counted in 10 non overlapping randomly chosen fields. The numbers obtained were analyzed using SPSS software version 16 (SPSS, Chicago, Illinois, USA).

Comparison between the different groups was made using unpaired T- test. The results were expressed as means ±SE. The differences were considered statistically significant when P values were less than 0.05.

## RESULTS

**H&E:**

Examination of H&E stained sections revealed that the myometrium is formed of hypertrophied smooth muscle fibers run in different orientations with congested blood vessels in both preterm and full term cases (**Figs. 1, 2**).

**Methylene blue:**

Telocytes appeared as branched cells with small oval cell bodies, flattened nuclei and thin long processes at the surface of smooth muscle fibers (**Figs. 3, 4**).

**Immunohistochemical results:**

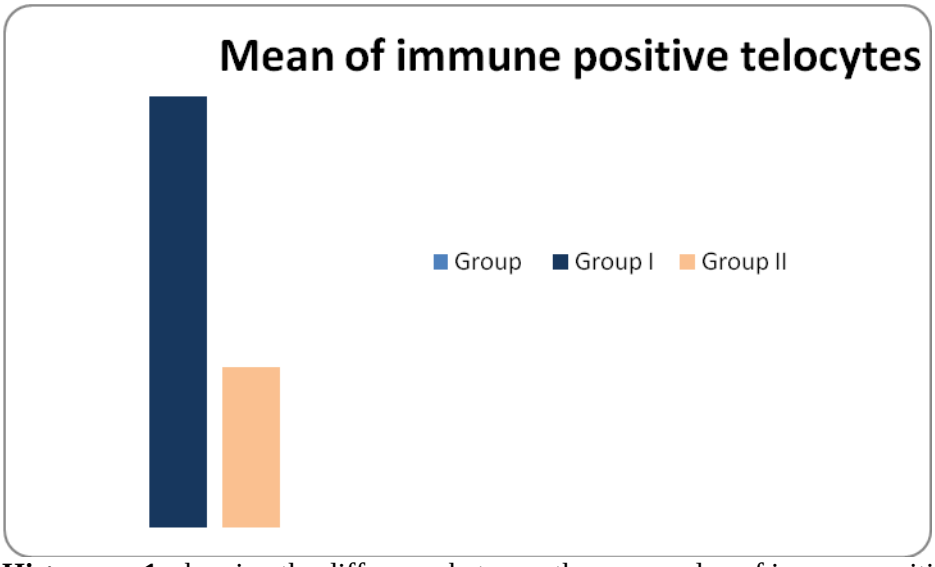
Examination of immune stained sections revealed an apparent numerous c-kit-positive cells in preterm cases compared to the full term ones (**Figs. 5,6**).

**Morphometric and statistical analysis**:

| Groups | Group I | Group II |
|---|---|---|
| Mean ±SE | 3.5±0.78 | 1.3±0.48 |
| P value | 0.000* | |

SE: Standard error

P value ≤ 0.05 significant




**Histogram 1**: showing the difference between the mean value of immunopositive telocytes in groups I and II

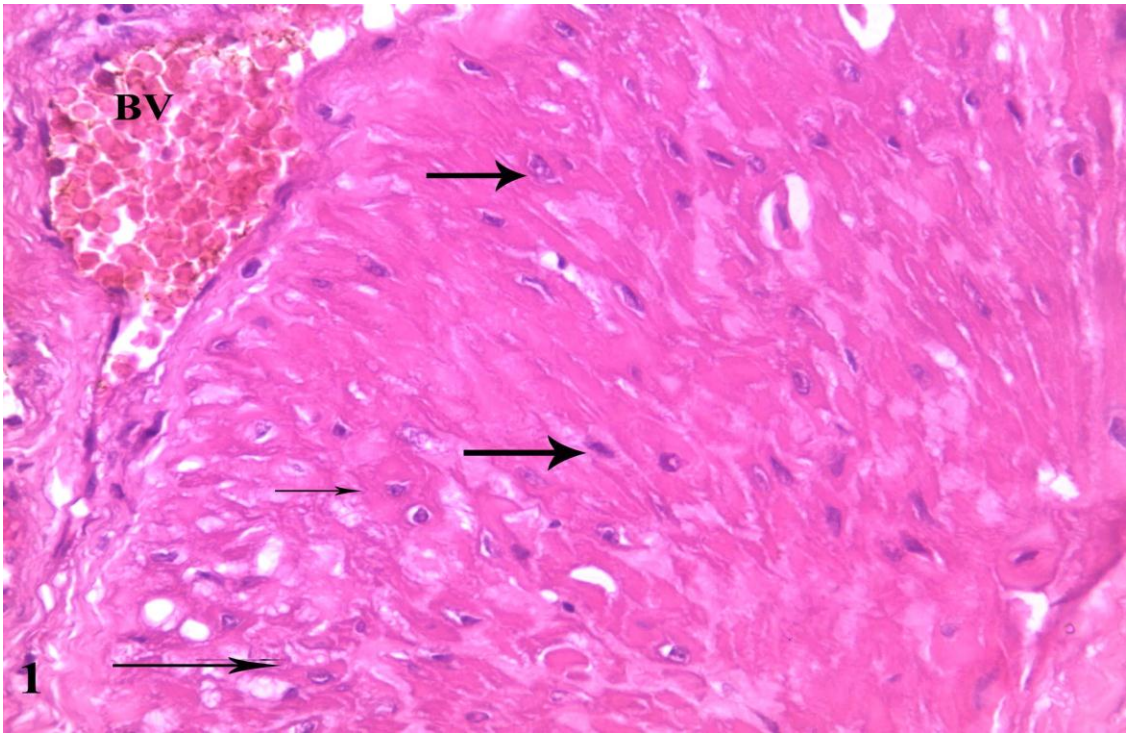



**Fig.1**: H&E stained section of group I showing hypertrophied smooth muscles in different directions (thick and thin arrows) with congested blood vessels (BV). (H&Ex400)

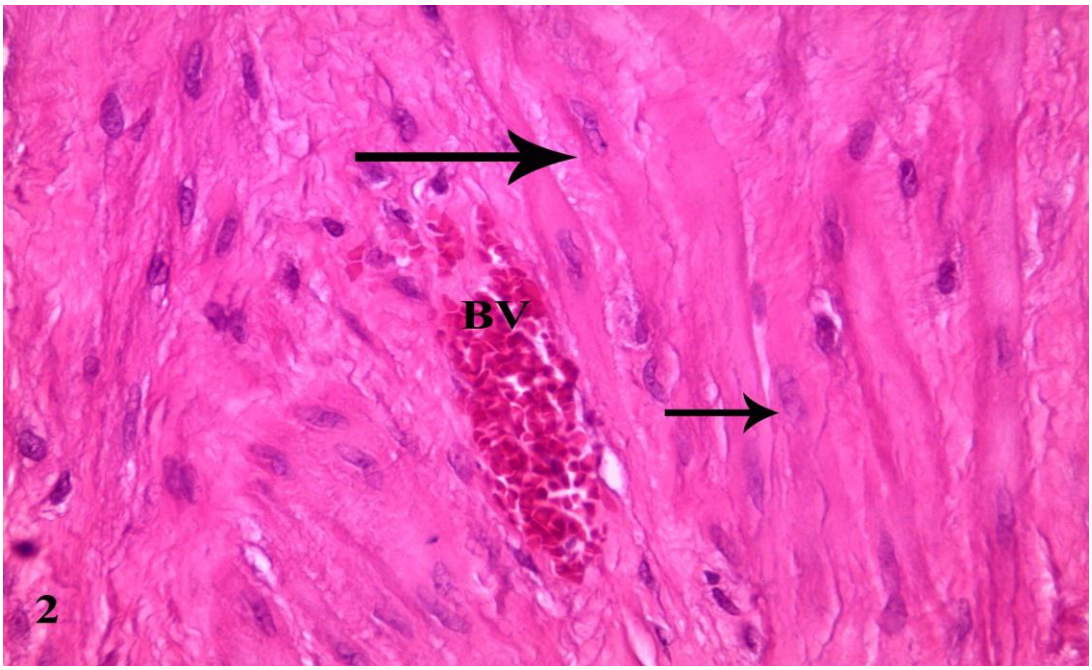



**Fig.2**: H&E section of group II showing hypertrophied smooth muscles in different directions (arrows) with congested blood vessels (BV). (H&Ex400)

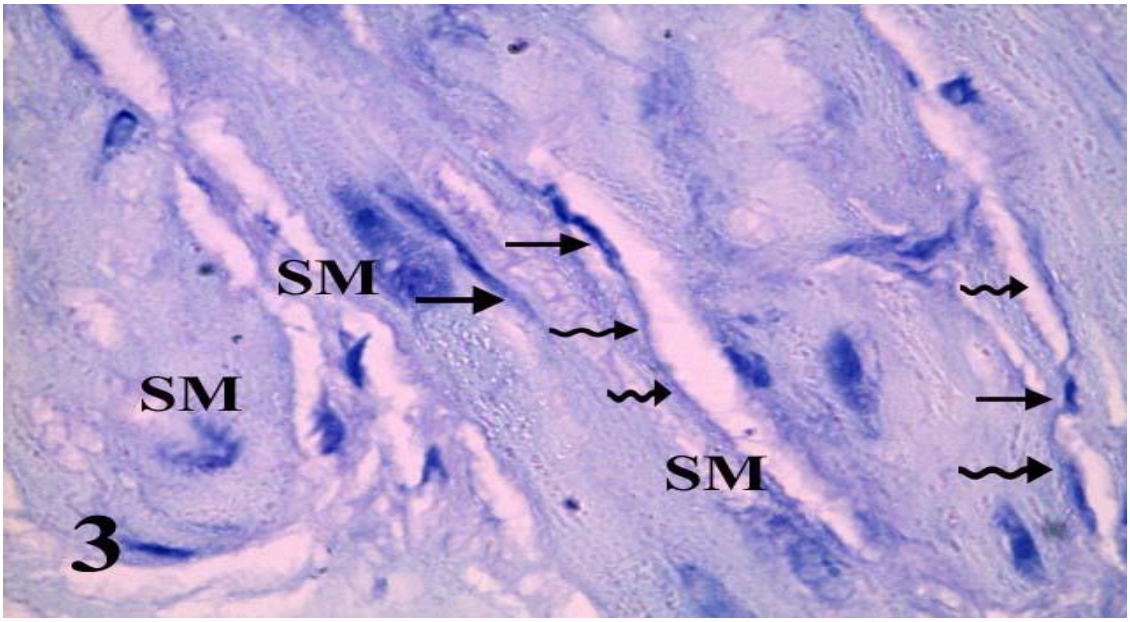



**Fig.3**: a photomicrograph of group I showing smooth muscle fibers (SM) and many telocytes (arrows). Note telocytes processes (irregular arrows) (Methylene blue x 1000)

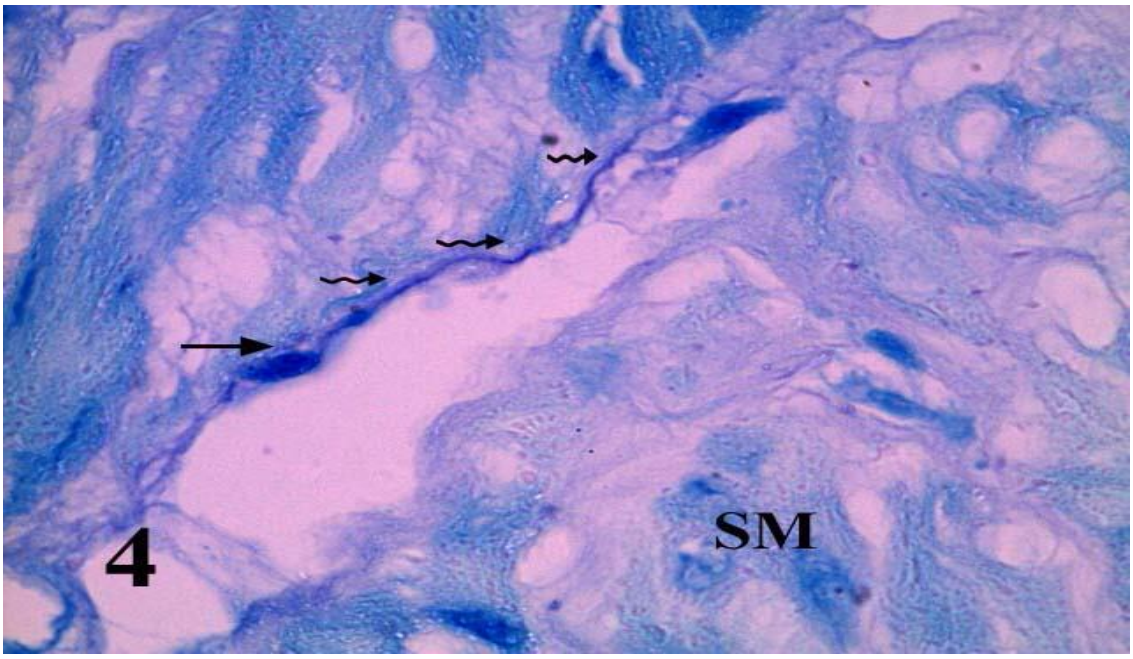



**Fig.4**: a photomicrograph of group II showing smooth muscle fibers (SM) and telocyte small cell body (arrow). Notice: telocyte long thin processes (irregular arrows) (Methylene blue x 1000)

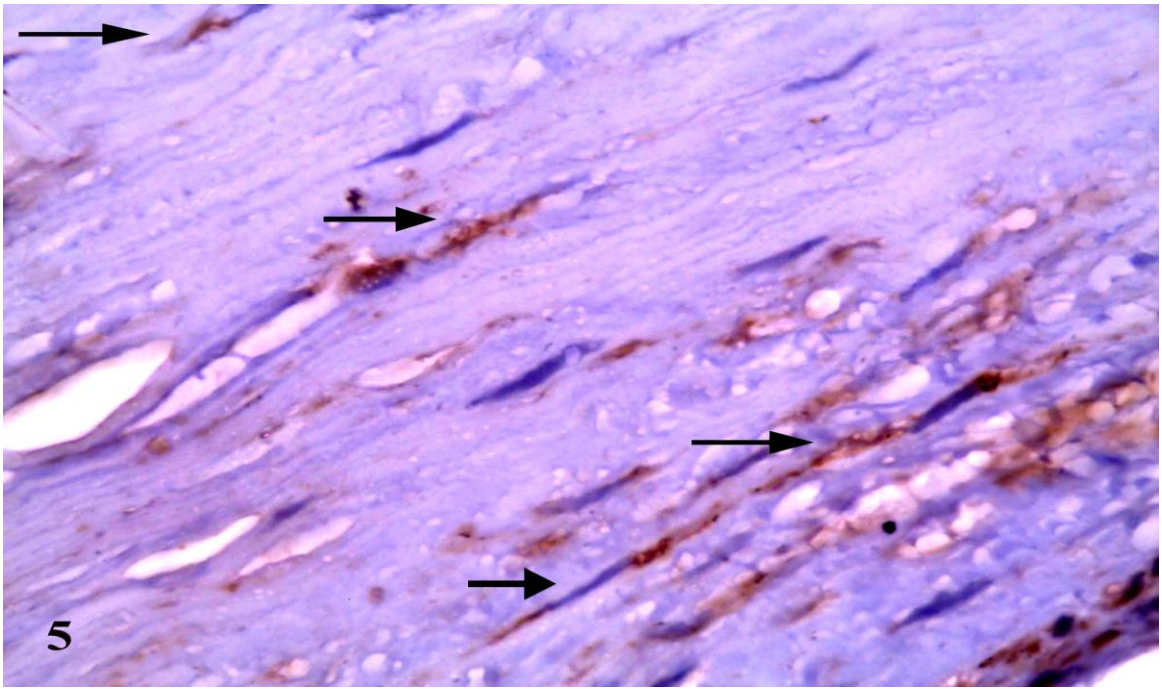



**Fig.5**: a photomicrograph of group I showing many immune positive telocytes between the smooth muscle fibers (SM). (C-kit immunostainingx1000)

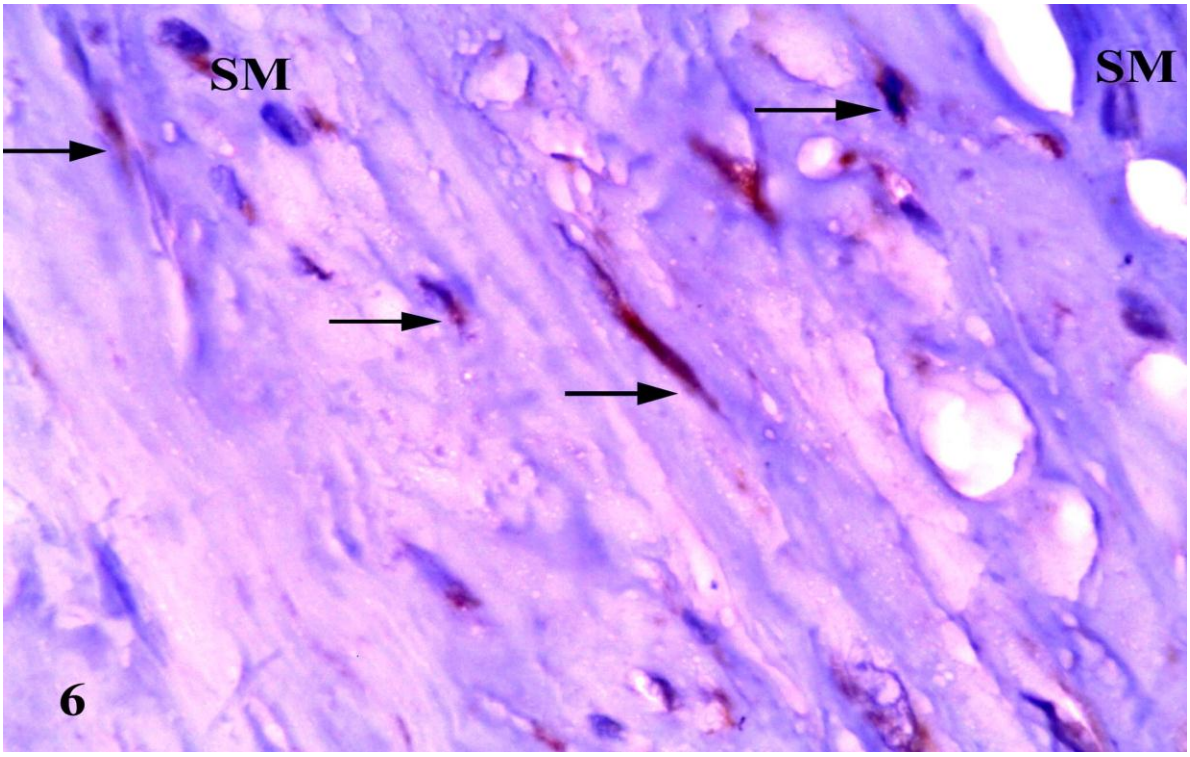



**Fig.6**: a photomicrograph of group II showing few telocytes (arrow) between the smooth muscles (SM). (C-kit immunostainingx1000)

## DISCUSSION

Preterm labour is a common obestetric problem. The discovery of TCs in the uterus raised its possible role in uterine physiology [19].

The present study was done to compare the number of telocytes in preterm and full term human pregnant uteri and the possible role of telocytes in preterm labour. Telocytes could not be identified in our study by routine H&E examination. So, methelyne blue and C-kit immunostaining were used to identify telocytes in the different groups [19, 23, 24].

In the present work we found a statistically significant higher number of telocytes in the myometrium of preterm cases compared to the full term ones. We hypothesize that increased number of telocytes could be a possible factor in preterm labour.

It is well known that uterine tissue has a complex physiology that is controlled by hormonal changes. Previous study reported that TCs are present in both the endometrium and the myometrium of rat's uterus. Pregnant uteri showed an increase in the endometrial TCs with a significant decrease in myometrial TCs compared to the non pregnant, which may possibly prevent preterm delivery [25]; this result is in accordance with the hypothesis of the present study. TCs in the endometrium supposed to have a role in glandular support, stromal cell communication and endometrial maintenance. In addition, they possibly initiate control and coordinate myometrial contractility. The highest count of myometrial TCs was recorded in the postpartum uteri on some studies and this result suggests their role in postpartum involution [25].

The role of TCs as pacemaking cells in the uterus is a matter of debate in contrast to pacemaking activity of Cajal cells in GIT which was well established [26]. Confirming this idea is the lack of regular slow waves of depolarization in TCs [6, 27].

Uterine contractility is modulated by hormones. TCs could act as uterine contractility modulator either by transferring bioactive molecules or by direct stimulation of SMCs as they express steroid hormone receptors [18, 28]. **Rehman *et al.*** [29] reported a switch from ERα to ERβ expression in the myometrium with the progression of pregnancy and this may delay labour until term. Furthermore, imatinib was found to inhibit the frequency and amplitude of

spontaneous myometrial contraction by interfering with TCs electrical modulator properties [20, 30, 31].

Telocytes have cell to cell contact via gap junctions between adjacent TCs and the smooth muscle cells [20]. In addition, their close relationships with capillaries, nerve endings and immune cells. These details can only be observed after two-dimensional reconstruction of successive microscopic TEM fields [6]. The contacts between TCs and immune cells (eosinophils, macrophages, and plasma cells) in rat myometrium may have a role in immune surveillance [32]. Leukocytes are known to be crucial for pregnancy maintenance and for the mechanism of uterine activation during labor. So, TCs, SMCs and leukocytes form a correlated orchestra that plays a role in the pregnancy maintenance or onset of labor [33,34, 35].

Telocytes were reported to have a particular ultrastructure in the form of a small cellular body with characteristic prolongations named telopodes which are composed of thin segments called podomers and dilated segments (podoms).It was reported that podoms were thicker in pregnant myometrium [6]. Telopodes have in their podoms calcium uptake/release units (caveolae, mitochondria and endoplasmic reticulum). The abundance of mitochondria in the podoms correlates with suggested hypotheses on the role of mitochondria as modulators in contractile activity in the mouse uterus [36].

Moreover, TCs express connexin 43, a gap junction protein, which most likely has a vital role in decidua maturation, as its decrease is associated with recurrent pregnancy loss [37].

TCs have been proposed as mechanoreceptors in rat mesentery [21]. The TCs are located in the human uterus at the border of SMCs and between them, thus TCs could be capable of detecting and translating stretch information to the nuclear factors and activate the genes responsible for protein synthesis [3]. Moreover, TCs from non pregnant and pregnant myometrium have different sensitivities to low-level laser stimulation and that mibefradil, a T-type calcium channel antagonist, can modulate this effect. Therefore, TCs act as a stretch sensor and may play an important role in the uterine contraction mechanism in a direct relationship with the pregnancy status [38].

**In conclusion**, up to our knowledge no previous studies upon telocytes in preterm and full term pregnant uteri were studied. Reviewing other studies with our findings we can say that telocytes may have a role in preterm labour not as pacemaker but as contractility modulator. Further numerous studies with electrophysiological investigation are needed for both endometrial and myometrial telocytes for more understanding uterine contractility in preterm labour.

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