

(RESEARCH ARTICLE)



Endometrial Stem cell count induced by Immuno-cyto labeling CD 105 and CD 90 marker staining in the healthy women individuals

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Abstract

Background: The current study was aimed to detect and localized of human endometrium stem cells at different age, through using CD105 and CD90 as stem cell markers.

Materials and methods: Fifty specimens of female adult endometrium from different age groups, including 20, 30, 40, 50, and 60, years were prepared for histological technique. Samples of uterine tissue were fixed in 10% buffer formalin and paraffinized as blocks, which were used for immunohistochemical studies localized and count the number of stem cells in endometrium of adult women. An immunohistochemical kit supplied by Pathn Situ PolyExel HPR/DAB detection system is used in present study.

Results: Expression of CD105 and CD90 given a clear result for the detection of endometrial stem cells (EnSCs). Results of the present study showed a highly expression count was found with CD90 more than with CD105. The localized expression was appeared in basalis stroma more than the functionalis. The means of CD90 expression in basalis were 45.20 ± 8.67 , 37.00 ± 5.69 , 34.60 ± 4.88 , 31.80 ± 4.38 and 30.60 ± 3.78 in I, II, III, IV and V age groups respectively. While in functionalis were 38.60 ± 9.21 , 37.40 ± 7.73 , 28.40 ± 4.04 , 25.00 ± 2.92 , 22.00 ± 2.12 in I, II, III, IV, V age groups. The results of expression values showed inverse relationship were decreasing with aged, and the mean cellular count of CD105 expression within basalis stroma was 44.20 ± 8.14 , 40.20 ± 9.98 , 34.20 ± 3.35 , 31.40 ± 4.34 , 30.40 ± 5.36 in I, II, III, IV, V age groups, otherwise the mean of CD105 expression in functionalis were 36.40 ± 6.43 , 35.40 ± 9.84 , 32.20 ± 4.76 , 26.20 ± 2.59 , 24.00 ± 3.94 of the same age groups. The results of CD 105 and CD 90 cellular markers in both basalis and functionalis stroma of endometrium in group I showed a significant difference when compared with other groups on level $P \leq 0.05$.

Conclusion: it was demonstrated in present study show the stem cells were decreasing in endometrium stroma gradually with increasing age. Expression rate of CD90 marker was more than CD105 on the other hand these markers showed a highly expression in basalis stroma than functionalis stroma

Keywords: CD105; CD90 cell markers; Immunohistochemistry; Uterine endometrial

1. Introduction

Stem cell is very rare in the adult individuals and have been detected within various adult tissues such as the skin [1], blood [2], nervous system [3], intestine [4], muscle [5], and endometrial uterine layer [6]. Basically, somatic stem cells (SSCs) or stem cells was a specialized cells having the ability to self-renewal and differentiated to specializing cell [7]. Stem cells were documented in several previous studies. during each menstruation cycle of women, the endometrium physiological changes was introduce into periodic event alterations such as renew, proliferative, differentiate, and shedding of [8], [9]. Furthermore these changes appeared post all pregnant and endometrial incisions [10] [11]. These changes of the uterine endometrial have pointed to low in number of endometrium somatic stem cell (SSC) populations which appeared to be responsible for its mention restoration effectively[12].

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Stem cells are specialized cells that can auto-renew, have the ability to divide into two new progeny stem cells, responsible for maintaining a cellular pool of stem cells within tissues of organs [13]. Human uterus undergoes two significant changes, these changes occur during the regular menstrual cycle and during pregnancy. To overcome these changes, it is suggested that stem cells play an important major role in maintaining and structural endometrium of uterus [14]. The CD90 molecule is expressed by CD34 cells in bone marrow which constitute about 10-40% of bone marrow cellular pool, The CD34+/CD90+ cells probably represent the most primitive hematopoietic progenitor cells and stromal cells [15]. CD105 is expressed on mesenchymal cells [16] and expressed in angiogenic endothelial cells (EC), vascular smooth muscle cells and fibroblasts [17]. Therefore recent study on stem cells, revealed the major roles of stem cells to renew tissues [18].

Aim of study

This study aimed to assess the existence and number of stem cells within the endometrium and recognize the variations of these cells between functional and basal layers at different ages.

2. Material and methods

2.1. Study design

Fifty specimens of adult healthy women were divided according to their female age into five groups Group I (20-29), group II (30-39), group III (40-49), group IV (50-59), group V (60-69) years respectively [19]. Current study was done in Azadi Teaching Hospital from May 2024 to August 2024.

Specimens of uterine endometrium were collected, post hysterectomy (uterine problems) during childbirth or from women registered in forensic medical center as a normal reason of death without any disease or damage in the uterus.

The uterus specimens were fixed with 10% buffered formalin for 24h, then fixed – specimens were paraffin embedded as paraffin block, then tissue specimens were then cut into 5-µm paraffin ribbon of tissue and used for the paraffin-immunohistochemistry technique (IHC) [20], was used to reveal stem cells within the endometrium by using two different markers (CD90 and CD105).

In immunohistochemistry kit used in current study was PolyExcel HRP/DAB Detected System two step universal kit for mice and rabbit primary antibodies (PathnSitu Biotechnologies/ India)

2.2. Procedure principal

De-paraffinized of tissue sample in 3 xylene changes, rehydration slides section by a series of descending alcohol. Incubate the sample tissues with 0.05 % H₂O₂ quenches any endogenous peroxidase enzyme activity for 5–10 minutes. Then the sample incubated with the adequate volume of dilute rabbit or mouse primary antibody, following by Poly incubation of Excel target binder for 10 minutes, then followed by incubation of PolyExcel HRP using provided for 10 minutes. The staining was performed through an incubation of 3,3'-diaminobenzidine (DAB) chromogenic substrate for 5–10 minutes, which give a brown- colored precipitate on the site of the antigen, immersion the sections with hematoxylin and incubate at room temperature for appropriate time, dehydrate sections in ascending alcohols and Xylene for clearing then mounting cover slip

3. Results

The results obtained from current study showed, endometrium tissue at during proliferative phase represent into two different layers; upper layer was functionalis and lower layer was basal which contains the basal parts of uterine glands. Depending on the obtained results from positive IHC- staining with CD90 and CD105 markers in both layers functionalis and basal as shown in fig (1, 2), the count of expression somatic stem cells (EnSCs) within the two represent layer functionalis and basal of the uterine endometrium, a highly expression for both CD90 and CD105 cellular markers with endometrium stem cells (EnSCs) at younger female age groups, and the expression of these markers was concentrated specifically within basal stem cells layer more than functionalis of endometrium layer, as shown in (Table1), and Fig (3, 4). The results of CD 105 and CD 90 cellular markers in both basal and functionalis layers of endometrium in group I showed a significant difference when compared with other groups on level $P \leq 0.05$ as shown in table 2 and 3. In comparison, the obtained data between CD90 and CD105 markers of endometrium stem cell expression with these markers uncovered that the expression of CD90 was highly more than the expression of cellular marker CD105 in all groups as shown in (table1, Fig3). these variations in the number of endometrium stem cells

expression with CD90 and CD105 cellular markers were highly in basalis layer more than the functionalis layer on the other hand the expression of cellular markers CD90 was highly expressed more than CD105 cellular markers in endometrial basalis layer.

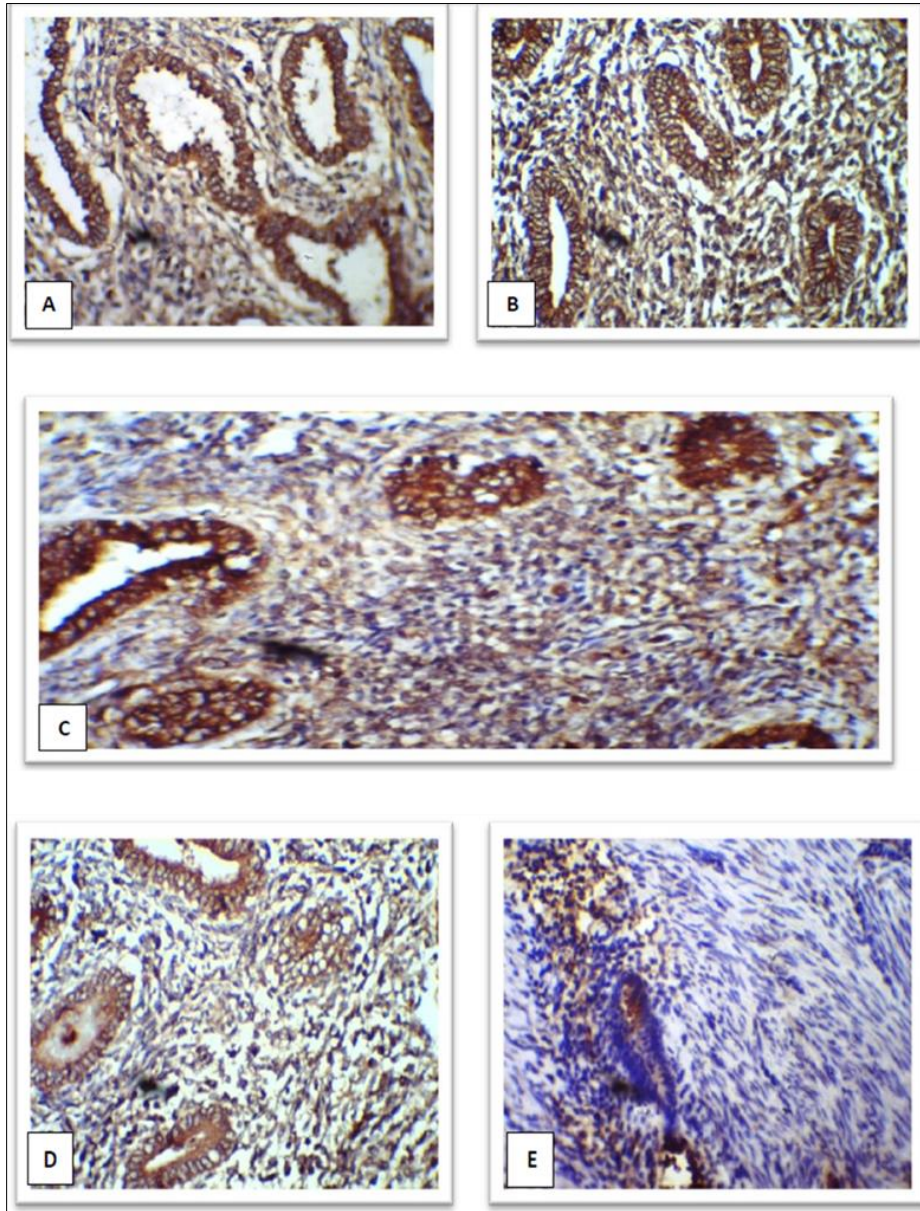


Figure 1 Endometrium sections showed expression of cellular marker CD90 in study groups ;group I(A); group II (B) ; group III (C); group IV (D) and group V (E). (x40)

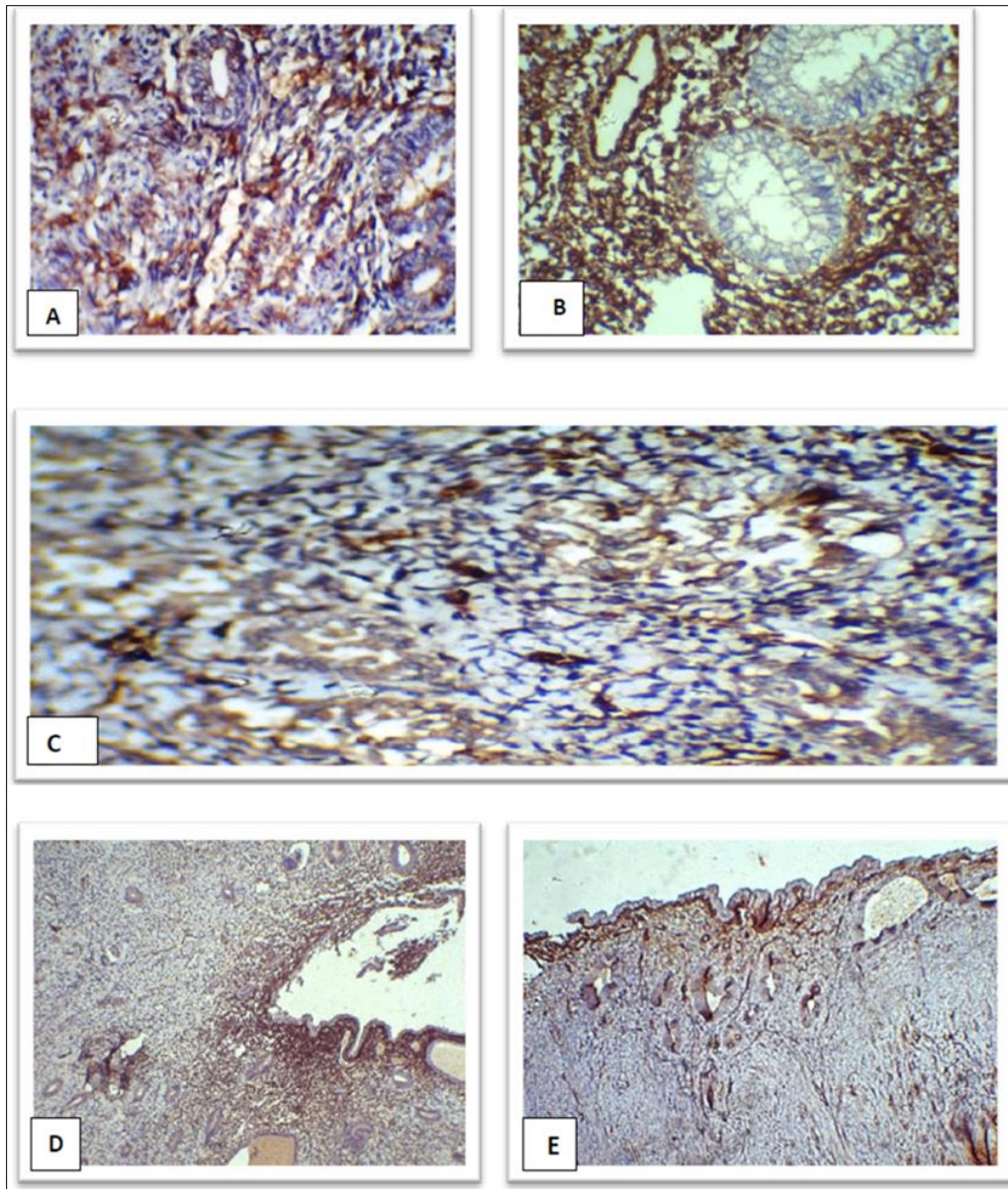


Figure 2 Endometrium sections showed expression of cellular marker CD105 in study groups ;group I(A); group II (B) ; group III (C); group IV (D) and group V (E). (x40)

Table 1 Positive stain with CD90 and CD105stem cell (SSCs) count in uterine endometrial layer basalis and functionalis

| study groups | CD90 | | CD105 | |
|--------------|-----------------------------|----------------------------------|-----------------------------|----------------------------------|
| | Basalis Mean±SD cell/hpf | Functionalis Mean±SD cell/hpf | Basalis Mean±SD cell/hpf | Functionalis Mean±SD cell/hpf |
| group I | 45.3 ±0.821 | 38.60±9.21 | 44.20±8.14 | 36.30±3.53 |
| group II | 38.00±6.69 | 37.00 ±5.32 | 40.30±2.08 | 35.40±9.84 |
| group II | 34.73±3 | 28.40±4.04 | 34.20±3.35 | 32.20±4.76 |
| group IV | 31.80±4.38 | 25.00±5.29 | 31.40±4.34 | 26.33 ±3.5 |
| group V | 30.60±3.78 | 22.20±1.6 | 30.40±5.36 | 24.00±3.94 |
| Total | 35.84±7.48 | 30.28±8.89 | 36.08±8.16 | 30.84±7.12 |

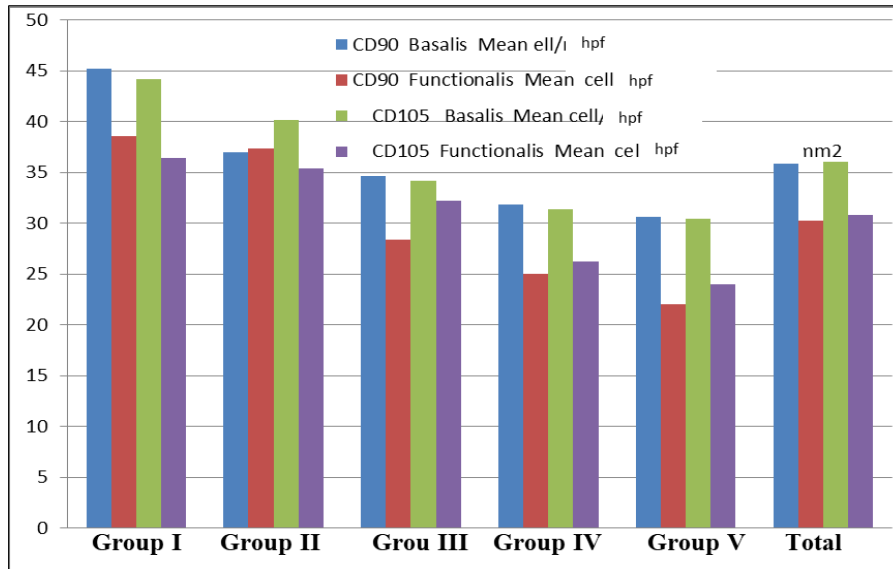


Figure 3 Showed the distributed CD90, CD105 cellular markers of endometrium stem cell (EnSCs) in both functionalis, basalis layer

Table 2 Positive stain with CD90 and CD105 stem cell (SSCs) count in uterine endometrial layer basalis and functionalis

| Study Group | CD 105 | | CD 90 | |
|-------------|-----------------|------------------|-----------------|-----------------|
| | basalis | functionalis | basalis | functionalis |
| G I(20-29) | 38.600A*9.209 B | 45.200*8.672 A | 36.400*6.427 B | 44.200A*8.136 B |
| GII (30-39) | 35.400*9.839 A | 37.000B *9.985 A | 38.400*7.733 A | 37.000* 5.958 A |
| G111(40-49) | 26.200*2.588 C | 31.200*3.347 B | 22.000* 2.121 D | 34.600*4.879 A |
| G IV(50-59) | 30.200*4.764 A | 33.400*4.336A | 25.000* 2.915 B | 31.800* 4.382 A |
| G V(60-69) | 26.000*3.937 B | 31.400*5.857 A | 27.400A*4.037 B | 30.600*3.782 A |

Table 3 Positive stain with CD105 and CD90 stem cell (SSCs) count in uterine endometrial layer basalis and functionalis

| Study Group | CD 105 | | CD 90 | |
|-------------|-----------------|-----------------|-----------------|------------------|
| | basalis | functionalis | basalis | functionalis |
| G I(20-29) | 36.400* 6.427 A | 44.200* 8.136 A | 38.600* 9.209 A | 45.200* 8.672 A |
| GII (30-39) | 35.400* 9.839 A | 40.200* 9.985 A | 38.400* 7.733 A | 37.000* 5.958 B |
| G111(40-49) | 26.200* 2.588 C | 31.200* 3.347 B | 22.000* 2.121 B | 34.600* 4.879 BC |
| G IV(50-59) | 30.200* 4.764 B | 33.400* 4.336 B | 25.000* 2.915 B | 31.800* 4.382 C |
| G V(60-69) | 26.000* 3.937 C | 31.400* 5.857 B | 27.400* 4.037 B | 30.600* 3.782 C |

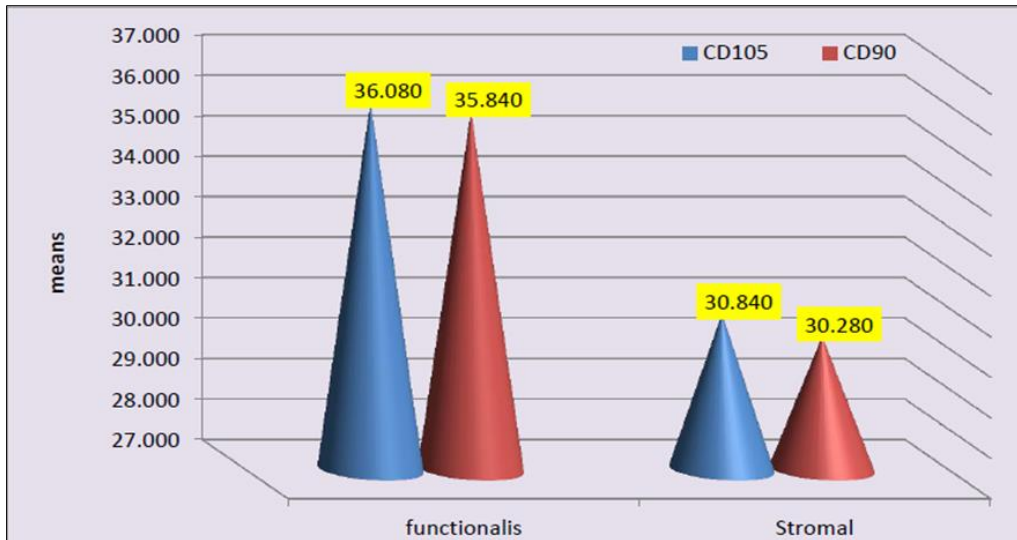


Figure 4 Showed the distributed CD90, CD105 cellular markers of endometrium stem cell (EnSCs) in both functionalis, basalis layer

4. Discussion

Stem cell was undifferentiated cell located in different adult tissues. Which responsible to homeostasis of organ, supplying alteration of cells in re- newal tissue which lost their cells by apoptosis or inflammations[21]. These providing role was highly controlled to reach in balance between stem cell replacement and save sufficient differentiated to mature cells in tissue and maintenance internal homeostasis of organ[22]. In present study was showed the appearance of stem cell within uterine endometrial of adults women. These results was in agreed with Prianiskiikov [23] who the first announced endometrial stem cells through deeper parts of basalis layer, with their regular differentiation was controlled by hormonal changes. As well as the present finding was in agreed Tempest et al [24] , whom pointed about the existence of endometrial stem cells is required because of highly regenerative endometrium submit about 4000 cycles of shedding of and regeneration over again during women's life.

The immunohistochemistry of current study showed a highly expression of CD90 cellular marker. these expression value localized in basalis layer more than the functionalis layer. The means of expression of cellular marker CD90 at group I (20-29) years in endometrial basalis and functionalis were 45.20 ± 8.67 and 38.60 ± 9.21 cells respectively. These density of both markers was highly in the younger age groups, but it decreases with age. Pathare and his colleagues proved, aging plays a major role in endometrium, through induced alterations that appeared at the nucleus, cellular, and histologic levels propose that aging has a negative impact on endometrium activity[25]. The results stem cells count revealed higher expression with CD90 cellular marker more than expression of CD105 cellular marker in all study groups. In other word the results showed difference in stem cells number when comparing between the two layers of endometrium the expression of stem cells with CD90 and CD105 cellular markers was highly in basalis layer more than functionalis. These expression are highly with CD90 more than with CD105 cellular marker. As has been reported from the current study results, the stem cells pool number are recorded in the basalis layer higher than the functionalis layer[26]. Study revealed the concentrate of endometrium stem cells in the basalis layer and inbetween uterine epithelial gland cells, Which responsible to give a signals to induced the renewal of the endometrium through menstruation cycle during women lifespans[27]. In addition, the intensive expression cell populations were located adjacent blood capillaries bed demonstrate significant increasing of progeny cells productions capability than Double-negative cell populations (immature cells lack CD4 and CD8) [28] , [29] . Fayazi, et al, [30], they declare during count stem cells population. They pointed out the fundamentals of expression of CD90 cellular marker was higher than the expression of CD105 cellular marker, their results are closer to our obtained results in current study. In current study, the intensive of both markers CD90 and CD105 was situated within endometrial basalis layer. This intensive was decreased with aged. Schwab and Gargett, [31] , are announced that, the endometrium stem cells of are localized in blood vessels as a pericytes. Another study, revealed that, CD90 cellular market and CD105 cellular market are represent as a novel markers of mesenchymal cells and act as localization marker of mesenchymal cell. Another study pionted a similar finding which revealed by Bozorgmehr, et al [32]the positive staining with CD90 and CD105 cellular markers expression was giving positive results in both layers of endometrium.

5. Conclusion

The following conclusion can be made from the results of present study; CD90 and CD105 cellular markers are stained the endometrium stem cells which concentrate within basalis and functionalis layer. The expression of CD90 cellular marker was highly than CD105 cellular marker at both layers basalis and functionalis of endometrium. Finally these intensity of expression of the two markers was decreased with age.

Compliance with ethical standards

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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