Journal of Ideas in Health

e ISSN: 2645-9248 Journal homepage: www.jidhealth.com Open Access

Original Article

# Detection of stem cells in human endometrium: immune-histochemical study

Sameeah Mejbel Hamad [1]*

## Abstract

In this work, we recruited CD105 and CD90 markers to identify the endometrial stem cells (EnSCs) in the human uterine endometrium. Fifty endometrial specimens have been extracted from twenty-five deceased women. All specimens have been embedded in paraffin block and prepared for immune-histochemical processing. The expression of CD105 and CD90 was counted per high power field (HPF). Paired sample t-test was used to compare the means between groups and age variables. The Statistical analysis was conducted using “SPSS16 software" The statistical significance was considered at less than 0.05. Results of paired sample t-test showed that the expression count (EC) at the Basalis layer (with CD105) was 5.26 points higher than the Functionalis layer (95% CI [3.43, 7.09]) and the correlation was strongly and positively related (r = 0.829, P < 0.001). The EC at Basalis layer (with CD90) was 4.96 points higher than Functionalis layer (95% CI [3.02, 6.89]) and the correlation was strongly and positively related (r = 0.746, P= < 0.001). The EC at Functionalis layer (with CD105) was 0.9 points higher than the Stroma layer (95% CI [2.27, 4.11]); however, the correlation was weak and positively related (r = 0.429, P = 0.032). The EC at Functionalis layer (with CD90) was 1.4 points higher than the Stroma layer (95% CI [2.1, 4.2]), but the correlation was moderately and positively related r = 0.547, P = 0.005). Findings of an independent-sample t-test showed that the EC of stem cells at the Functionalis, Basalis, and Stroma layers (with CD105 and CD90 markers) was more among patients of reproductive age (<50 years) than patients who were at non-reproductive age (50 years and above), a statistically significant difference [(m = 9.6, 95% CI (5.3, 14.6), t (19.630) = 4.413, P < 0.001)], [(m = 10.2, 95% CI (5.2, 15.2), t (20.714) = 4.202, P < 0.001)] and [(m = 8.4, 95% CI (3.5, 13.4), t (12.313) = 3.523, P = 0.002)], respectively. In conclusion, the EC of the stem cells in the endometrium decreases with age.

**Keywords:** Stem cell, Immunohistochemistry, Uterine Endometrium, CD90, CD105, Iraq

## Background

Multipotent stem cells are infrequent in the adult stage that has been specified in different adult tissues such as the intestine [1], skin [2], muscle [3], blood [4], and nervous system [5], and endometrium [6]. In general terms, stem cells or somatic stem cells (SSCs) are specialized cells that can self-renew and give rise to differentiated cells [7]. Stem cells were reported in many previous studies. The endometrium physiologically undergoes cyclical changes such as self-renewal, reproduction, differentiation, and shedding during each woman's menstrual cycle. [8,9]. Furthermore, endometrial renewal occurs after all endometrial incisions and pregnancy [10,11]. These features of the endometrium have indicated the being of a low number of endometrial-derived stem cell (EnSC) populations that appear to be accountable for its noteworthy restoration potency [12]. Stem cells are specialized cells having the capability to self-renewal and have the ability to produce two new identical daughter stem cells for the maintenance of a pool of stem cells in the tissue [13]. The gradually occurring physiological changes in females, especially those bearing children, make them more vulnerable to emotional exhaustion [14]. The human uterus undergoes two remarkable changes. The first is during regular cyclic change of the menstrual cycle, and the second is during pregnancy. The stem cells are essential in replacing and maintaining uterine endometrial structure [15]. Recently published work about the biology of stem cells proved that the renewal of the damaged tissue is made by progenitor or stem cells [16]. This study aimed to identify the endometrial stem cells (EnSCs) and the likelihood of variations in different layers of human uterine endometrium using the CD105 and CD90 markers, respectively.

*Correspondence: samieaalgenabi@gmail.com
[1]Department of Anatomy, College of Medicine, Anbar University, Anbar, Iraq.

A full list of author information is available at the end of the article.

## Methods

### Study design

Prospective immunohistochemistry research was designed to collect convenient "uterine samples" of recently deceased women in Iraq from April 2018 to June 2019. Based on Immunohistochemistry (IHC) Handbook [17], "CD90" and "CD105 markers" were recruited to detect stem cells in the endometrium.

### Inclusion and exclusion criteria

All Iraqi women, regardless of age and marital status, whose deaths were recorded in forensic medicine due to natural causes, considering the absence of diseases or damage to the uterine tissue, have been included. However, we excluded the known samples with uterine problems during childbirth, such as severe bleeding or severe damage to uterine tissue due to accidents or chronic space-occupying lesions or diseases.

### Procedure of study

In this study, we performed an Immunohistochemical analysis of paraffin-embedded sections, IHC(P). The clinical and research laboratories use the standard technique known as Paraffin embedding to create a formalin-fixed, paraffin-embedded tissue block (FFPE). To reduce the appearance of a back floor on the tissues, both "PathnSitu's highly sensitive" and "specific PolyExcel two-step detection system" has been adopted. The first step is the deparaffinization of tissue pieces in 3 xylene shifts considering hydrating slides to water in a series of alcohol gradations.

The specimen should be incubated with 0.5% H2O2 for 5–10 minutes at room temperature to quench the endogenous peroxidase activity. Then coupling the specimen with the appropriate diluted mouse or rabbit primary antibody, followed by incubation with the PolyExcel target binder for 10 minutes, then another 10 minutes of incubation by a polymer labeled PolyExcel HRP.

The next step was the staining procedure. The general technique was to incubate the specimen with 3,3'-diaminobenzidine (DAB) chromogenic substrate for 5–10 minutes, giving a brown-colored precipitate at the antigen site. To prepare the working solutions, add "1 drop of Stunn DAB chromogen in a1ml of Stunn DAB buffer, mix well and store it at 2-8 Co in the dark medium. The prepared solution is stable for a week; however, it should always be prepared fresh for smooth and crisp results".

### Statistical analysis

The immunostained cells per nucleated cell were counted directly in the cassette under a fluorescent microscope. The number of immunostained cells was observed and recorded per high power field (HPF). Statistical analysis was conducted using SPSS16 software. The quantitative results were presented as means ± standard deviation. The difference in means was compared by Paired sample t-test between groups Pearson correlation test. An independent t-test was recruited to determine differences in the expression count of stem cells among the endometrial layers (Functionalis, Basalis, Stroma) with sociodemographic factor (age). The statistically significant is considered at less than 0.05.

## Results

### Out Sociodemographic factors

Twenty-five uterine samples have been extracted from deceased women. The mean age was 40.2 (SD 5.7). The age variable was further categorized (based on mean value) into the reproductive age group (<50 years old) and the non-reproductive age group (50 years and above).

### The EC of stem cells at the Functionalis and Basalis layers with CD105 markers

The Paired Samples t-test was run to compare the expression count of stem cells at the functionalis and basalis layers of the uterine endometrium with CD105 markers. Functionalis and Basalis scores were strongly and positively correlated ($r = 0.829$, $P < 0.001$). There was a significant average difference between Functionalis and Basalis scores ($t24 = 5.933$, $P < 0.001$). Basalis scores were 5.26 points higher than Functionalis scores (95% CI [3.43, 7.09]).

### The EC of stem cells at the functionalis and basalis layers with CD90 markers

The Paired Samples t-Test was run to compare the expression count of stem cells at the Functionalis and Basalis layers of the uterine endometrium with CD90 markers. Functionalis and Basalis scores were strongly and positively correlated ($r = 0.746$, $P < 0.001$). There was a significant average difference between Functionalis and Basalis scores ($t24 = 5.283$, $p < 0.001$). Basalis scores were 4.96 points higher than Functionalis scores (95% CI [3.02, 6.89]).

### The EC of stem cells at the functionalis and Stroma layers with CD105 markers

The Paired Samples t-test was run to compare the expression count of stem cells at the Functionalis and Stroma layers of the uterine endometrium with CD105 markers. Functionalis and Stroma scores were weakly and positively correlated ($r = 0.429$, $P = 0.032$). There was no significant average difference between Functionalis and Stroma scores ($t24 = 0.594$, $P = 0.558$). On average, Stroma scores were 0.9 points higher than Functionalis scores (95% CI [2.27, 4.11]).

### The EC of stem cells at the functionalis and Stroma layers with CD90 markers

The Paired Samples t-test was run to compare the expression count of stem cells at the functionalis and Stroma layers of the uterine endometrium with CD90 markers. Functionalis and Stroma scores were moderately and positively correlated ($r = 0.547$, $P = 0.005$). There was no significant average difference between Functionalis and Stroma scores ($t24 = 0.686$, $p < 0.499$). On average, Stroma scores were 1.4 points higher than Functionalis scores (95% CI [2.1, 4.2]). An independent-sample t-test was run to determine if there were differences in the EC of stem cells at the functionalis, Basalis, and Stroma layers (with CD105 and CD90 markers) between patients aged less than fifty years (reproductive age) and patients aged fifty years and above (non-reproductive age). Overall, the EC of stem cells at the Functionalis (with CD105 and CD90 markers) was more among patients of reproductive age (<50 years) ($m = 35.5$, $SD = 5.5$) than patients who were of non-reproductive age (50 years and above) ($m = 25.9$, $SD = 5.6$), a statistically significant

difference (m = 9.6, 95% CI (5.3, 14.6), t (19.630) = 4.413, p < 0.001). Overall, the EC of stem cells at the Basalis (with CD105 and CD90 markers) was more among patients of reproductive age (<50 years) (m = 41.3, SD = 5.6) than patients who were of non-reproductive age (50 years and above) (m = 31.1, SD = 6.2), a statistically significant difference (m = 10.2, 95% CI (5.2, 15.2), t (20.714) = 4.202, p < 0.001). Overall, the EC of stem cells at the Stroma (with CD105 and CD90 markers) was more among patients of reproductive age (<50 years) (m = 35.9, SD = 7.8) than patients who were of non-reproductive age (50 years and above) (m = 27.5, SD = 4.1), a statistically significant difference (m = 8.4, 95% CI (3.5, 13.4), t (12.313) = 3.523, p = 0.002).

**Table 1:** Paired sample t-test to compare the expression count of stem cells at the different endometrial layers (n=25)

| **Factors** | Category | Mean (SD) | *R-value | P-value | Mean difference (SD) | T-test | C.I.95% | P-value |
|---|---|---|---|---|---|---|---|---|
| **CD105** | Basalis | 35.2(7.7) | 0.829 | <0.001 | 5.3(4.4) | 5.933 | 3.43-7.09 | <0.001 |
| | Functionalis | 29.9(7.4) | | | | | | |
| **CD90** | Basalis | 34.2(6.8) | 0.746 | <0.001 | 4.9(4.7) | 5.283 | 3.02-6.89 | <0.001 |
| | Functionalis | 29.3(6.3) | | | | | | |
| **CD105** | Stroma | 30.8(7.1) | 0.429 | 0.032 | 0.9(7.7) | 0.594 | 2.27-4.11 | 0.558 |
| | Functionalis | 29.9(7.4) | | | | | | |
| **CD90** | Stroma | 30.3(8.9) | 0.547 | 0.005 | 1.4(7.6) | 0.686 | 2.1-4.2 | 0.499 |
| | Functionalis | 29.2(6.3) | | | | | | |

## Discussion

Adult stem cells are uncommon undifferentiated cells existent in various adult tissues. Stem cells play an important role in tissue homeostasis, altering cells to renew tissues lost by apoptosis or injury [18]. The role of stem cells is highly arranged to ensure a convenient balance in stem cell replacement and save sufficient differentiated mature cells for tissue and organ function [19]. The present work proved the existence of stem cells in the endometrium of the adult uterus. These findings agreed with Prianiskiikov's study [20]. The author was the first to believe the extended endometrial adult stem cells in the deeper Basalis layer, with their differentiation marked by functional changes in hormonal capability.

Moreover, Tempest et al. [21] indicated that endometrium stem cells were necessary because the human endometrium is a highly regenerative organ, submit over 4000 cycles of shedding and regeneration over a woman's lifetime. Our study showed a high expression with the CD105 and CD90 markers at the Basalis layer compared to the Functionalis. A similar finding has been reported by Fayazi et al. [12]. The authors used a scoring system to record their results. They found that the core of expression with CD90 was more than the expression with CD105. Their results are similar to the results obtained in the present work. Our findings showed that the intensity of the two markers CD105 and CD90 was higher at the endometrial Basalis, Functionalis and Stroma layers of patients aged less than forty years (reproductive age group) than patients aged forty and above (non-reproductive age).

Moreover, the intensity mentioned above significantly decreased with increasing age. Similarly, Schwab and Gargett [22] have recorded that the stem cells of the endometrium are located in vessels in the perivascular cells. In another study, it has been proved that CD90 and CD105 are considered mesenchymal key markers to characterize mesenchymal stem cell localization. Also, a similar result was obtained by Schwab et al. [23] that CD90 and CD105 markers expression stained the two layers of endometrial Functionalis and Basalis. This study suffers from some limitations. Some of the limitations are related to routine procedures in forensic medicine institutions, which caused the study time to be prolonged. Some others are related to the difficulty of obtaining the consent of the deceased's relatives due to legal and social reasons.

## Conclusion

In conclusion, the expression count (EC) with CD105 and CD90 markers was 5.26 and 4.96 points higher in Basalis than in Functionalis. While the expression count (EC) with CD105 and CD90 markers was 0.9 and 1.4 points higher in Functionalis than Stroma, respectively. Moreover, Functionalis, Basalis, and Stroma layers of the patients in the reproductive age group showed high expression count (EC) with CD105 and CD90 markers compared to patients aged forty years old or above (non-reproductive age), respectively.

### Abbreviation

EnSCs: Endometrial Stem Cells; HPF: High Power Field: EC: Expression Count; SSCs: Stem Cells or Somatic Stem Cells; IHC: Immunohistochemistry; FFPE: Paraffin-Embedded Tissue Block; DAB: Diaminobenzidine

### Declaration

### Acknowledgment

We would like to thank all the deceased women's relatives for their understanding, cooperation, and support of the study. Also, our thanks to the directors and workers in the forensic centers for their unlimited support during the sample collection process.

### Funding

The authors received no financial support for their research, authorship, and/or publication of this article.

### Availability of data and materials

Data will be available by emailing samieaalgenabi@gmail.com.

### Authors' contributions

Sameeah Mejbel Hamad (SMH) is the corresponding and the responsible author of the concept, design, procedure, data analysis and data acquisition, manuscript writing, editing, and reviewing. The author (SMH) has read and approved the final manuscript.

## Ethics approval and consent to participate

The author conducted the research following the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Scientific Issues and Postgraduate Studies Unit (PSU), College of Medicine, University of Anbar (Ref: 3/7/451 at 07-March-2018). Moreover, informed consent was obtained from each deceased's relative after explaining the study objectives and the guarantee of secrecy.

## Consent for publication

Not applicable

## Competing interest

The authors declare that they have no competing interests.

## Open Access



## Author Details

[1]Department of Anatomy, College of Medicine, Anbar University, Anbar, Iraq.

## Article Info

Received: 12 April 2022
Accepted: 13 May 2022
Published: 14 May 2022

## References

1. Cervelló I, Santamaría X, Miyazaki K, Maruyama T, Simón C. Cell Therapy and Tissue Engineering from and toward the Uterus. Semin Reprod Med. 2015 Sep;33(5):366-72. doi: 10.1055/s-0035-1559581.
2. Alonso L, Fuchs E. Stem cells of the skin epithelium. Proc Natl Acad Sci U S A. 2003 Sep 30;100 Suppl 1(Suppl 1):11830-5. doi: 10.1073/pnas.1734203100.
3. Jankowski RJ, Prantil RL, Fraser MO, Chancellor MB, De Groat WC, Huard J, Vorp DA. Development of an experimental system for the study of urethral biomechanical function. Am J Physiol Renal Physiol. 2004 Feb;286(2): F225-32. doi: 10.1152/ajprenal.00126.2003.
4. Eaves CJ. Hematopoietic stem cells: concepts, definitions, and the new reality. Blood. 2015 Apr 23;125(17):2605-13. doi: 10.1182/blood-2014-12-570200.
5. Wang YZ, Plane JM, Jiang P, Zhou CJ, Deng W. Concise review: Quiescent and active states of endogenous adult neural stem cells: identification and characterization. Stem Cells. 2011 Jun;29(6):907-12. doi: 10.1002/stem.644.
6. Gargett BE, Chan RW. Endometrial stem/progenitor cells and proliferative disorders of the endometrium. Minerva Ginecol. 2006 Dec;58(6):511-26.
7. Maruyama T. Endometrial stem/progenitor cells. Journal of Obstetrics and Gynaecology Research. 2014 ;40(9):2015-22.
8. Chan RW, Schwab KE, Gargett CE. Clonogenicity of human endometrial epithelial and stromal cells. Biol Reprod. 2004 Jun;70(6):1738-50. doi: 10.1095/biolreprod.103.024109. Epub 2004 Feb 6.
9. Gargett CE. Identification and characterisation of human endometrial stem/progenitor cells. Aust N Z J Obstet Gynaecol. 2006 Jun;46(3):250-3. doi: 10.1111/j.1479-828X.2006.00582. x.
10. Gargett CE, Ye L. Endometrial reconstruction from stem cells. Fertil Steril. 2012 Jul;98(1):11-20. doi: 10.1016/j.fertnstert.2012.05.004. Epub 2012 May 30.
11. Gargett CE. Uterine stem cells: what is the evidence. Human reproduction update. 2007;13(1):87–101. https://doi.org/10.1093/humupd/dml045
12. Fayazi M, Salehnia M, Ziaei S. Characteristics of human endometrial stem cells in tissue and isolated cultured cells: An Immunohistochemical Aspect. Iranian biomedical journal 2016; 20(2):109.
13. Ramalho-santos M, Willenbring H. On the origin of the term ″stem cell″. cell stem cell. 2007;1(1):35-38.
14. Dastan I, Al-samarraie M, Ali Jadoo SA. Female doctors are more emotionally exhausted than their male counterparts in Iraq. jidhealth [Internet]. 29Jun.2019 [cited 18Sep.2021];2(1):75-9. doi: 10.47108/jidhealth.vol2.iss1.18
15. Ohagwu CC, Agwu KK, Abu PO. Real time sonographic assessment of common uterine sizes, shapes and positions in Nigerians. J Expt Clin Anat 6.1 (2007);6(1) :41-6.
16. Escalante NMM, Pino JH. Arrangement of Muscle Fibers in the Myometrium of the Human Uterus: A Mesoscopic Study. MOJ Anatomy & Physiology.2017;4(2):131-135.
17. Immunohistochemistry (IHC) Handbook, Novus Biological. Available from: https://images.novusbio.com/design/BR_IHCGuide_011017_web.pdf
18. Li L, Xie T. Stem cell niche: structure and function. Annu Rev Cell Dev Biol. 2005; 21:605-31. doi: 10.1146/annurev.cellbio.21.012704.131525.
19. Moore KA and Lemischka IR. Stem cells and their niches. Science,2006; 311,1880–1885.
20. Prianishnikov VA. On the concept of stem cell and a model of functional- morphological structure of the endometrium. Contraception,1978; 18,213–223.
21. Tempest N, Maclean A, Hapangama DK. Endometrial Stem Cell Markers: Current Concepts and Unresolved Questions. Int J Mol Sci. 2018 Oct 19;19(10):3240. doi: 10.3390/ijms19103240.
22. Schwab KE, Gargett CE. Co-expression of two perivascular cell markers isolates mesenchymal stem-like cells from human endometrium. Hum Reprod. 2007 Nov;22(11):2903-11. doi: 10.1093/humrep/dem265.
23. Schwab KE, Hutchinson P, Gargett CE. Identification of surface markers for prospective isolation of human endometrial stromal colony-forming cells. Hum Reprod. 2008 Apr;23(4):934-43. doi: 10.1093/humrep/den051.