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ORIGINAL RESEARCH

A Preliminary Study on the Correlation Between Age and Endometrial Receptivity

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Background: It is well established that female fertility declines with age, primarily because of loss of ovarian function. However, few studies have clarified the relationship between increasing age and endometrial receptivity. Here, we aimed to study the impact of age on endometrial receptivity, meanwhile, we examined the expression of endometrial mesenchymal stem cell (eMSC) surface markers (CD146 and PDGF-Rβ), essential for endometrial development and re-growth, in different age groups.

Methods: Participants were enrolled in this study between October 2020 and July 2021. All 31 patients were divided into three age groups; early (30–39 years old, n=10), intermediate (40–49 years old, n=12) and advanced (≥50 years old, n=9). We assessed localization and expression of CD146 and PDGF-Rβ by immunofluorescence and further analyzed selected endometrial receptivity markers (Homeobox A10 HOXA10, leukemia inhibitory factor LIF and osteopontin) and steroid hormone receptors by immunohistochemistry.

Results: There were no significant differences in expression of HOXA10 and OPN ($p>0.05$) among the three groups. However, we found a significant difference in LIF expression between the early and advanced age groups, with higher expression noted in the latter group ($p=0.02$). Similarly, estrogen receptor (ER) and progesterone receptor (PR) expression were significantly increased ($p=0.01$ and $p=0.01$, respectively) in the advanced age group compared with the early age group. There were no significant difference in CD146 and PDGF-Rβ expression among the three groups ($p>0.05$).

Conclusion: These results suggest that the age of the patient does not influence their endometrial receptivity. So, this study serves to increase our understanding of the impact of age and eMSCs on endometrial receptivity and expands the etiology of age-related infertility.

Keywords: age, endometrial receptivity, endometrial mesenchymal stem cells

Introduction

It is estimated that the global incidence of female infertility lies between 8% and 12%,[1] this incidence may be an underrepresentation given the continuing increase in delayed childbearing over time.[2] Research indicates that female fertility declines with age,[3] starting at age 32, followed by a more rapid decrease after age 37 years.[4] This decline in fertility is related to diminished ovarian reserve including reduced oocyte quality and number.[5] Embryo implantation, which is essential for successful pregnancy, depends on "cross-talk" between the embryo and the endometrium. This process involves two key elements: embryo quality and endometrium functional status. Defects in either may result in implantation failure.[6] The state of the endometrium when the endometrial epithelium is suitable for embryo implantation is labelled the "phase of receptivity".[7] It is accepted that egg quality declines with age;[8] however, whether endometrial receptivity also declines with age is still somewhat controversial.[9] Currently, there is little research in this area.

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Embryo implantation, blastocyst apposition, adhesion and invasion are key steps in determining whether a pregnancy will develop successfully. Hence, it is useful for us to understand critical aspects of this window period. A number of molecules have been recognized as endometrial receptivity markers, including HOXA10, LIF, osteopontin (OPN) and integrin αvβ3,[10–13] which are used for the evaluation and analysis of endometrial function.

Adult stem cells (ASCs), identified in rodent and human endometria, play an important role in endometrial development and re-growth and can differentiate into stratum functionalis glandular and stromal tissue.[14] Endometrial mesenchymal stem cells (eMSC), an important subtype of ASCs, are identifiable by markers CD146 and PDGF-Rβ.[15] By studying them, we attempted to gauge progression of endometrial pathology and characterize the existence of endometrial stem cells in patients of differing ages during the implantation period.

Thus, the primary aim of this study was to determine whether endometrial receptivity declines as women age. Second, we further analyzed endometrial stem cell surface markers in different age groups, to determine whether it is a major factor affecting endometrial receptivity.

Materials and Methods

Patient Selection

All patients were admitted to the Department of Gynecology and Obstetrics, the First Affiliated Hospital of Shandong First Medical University for hysterectomy and hysteroscopic surgery between October 2020 and July 2021. Thirty-one (31) patients were randomly divided into three groups according to age; early (30–39 years old, n=10), intermediate (40–49 years old, n=12) and advanced (≥50 years old, n=9) age groups (Table 1). Exclusion criteria were: (1) Endometrial pathology such as polyps, submucosal uterine fibroids or other conditions likely to impact endometrial receptivity; (2) Endocrine therapy including oral contraception, gonadotropin-releasing hormone agonists, or hormone replacement therapy for at least 6 months before this study. Informed consent was obtained from all patients. Endometrial tissue samples were collected from total hysterectomy or hysteroscopic surgical procedures. The patients underwent hysteroscopy for examination and removal of an intrauterine device. In addition, the patients underwent hysterectomy due to uterine prolapse and cervical cancer. All of the patients participated, intraoperative and postoperative pathological findings indicated the endometrium was normal. They all have a regular menstrual cycle for premenopausal patients. For them, all samples were obtained from the middle secretory phase of the menstrual cycle (D20–23). For postmenopausal patients, the samples were collected randomly.

The study was approved by the Ethics Committee of The First Affiliated Hospital of Shandong First Medical University. All patients signed written consent to participate according to the Declaration of Helsinki. All participants were informed about the purpose of the study.

Hematoxylin and Eosin Staining

Endometrial tissues were immediately fixed with formalin and embedded in paraffin. Paraffin-embedded blocks were serially sectioned at 5 μm and mounted slides stained with hematoxylin and eosin. Slides were analyzed using light microscopy (Zeiss, Oberkochen, Germany). Three experienced observers independently assessed the morphology and structure of endometrium.

Table 1 Age Distribution of 31 Patients for This Study

Group	Age (Years)	n	%
Early age group	30–39	10	32.26
Intermediate age group	40–49	12	38.70
Advanced age group	≥50	9	29.04
	Total	31	100

Immunohistochemistry

Paraffin-embedded sections were deparaffinized in xylene and rehydrated by sequential incubation in absolute ethanol dilutions. Antigen retrieval was performed by immersing sections in citric acid buffer (pH 6.0) in a microwave oven for 15 min. The sections were incubated with 3% H_2O_2 for 25 min at room temperature to eliminate endogenous peroxidase activity, and then blocked with 3% Bovine Serum Albumin (BSA) for 30 min at room temperature. The slices were incubated overnight at 4°C with the following primary antibodies: HOXA10 (Abcam, ab191470;1:100), LIF (Wuhan Servicebio Technology, GB121477, 1:2000), Osteopontin (Abcam, ab214050;1:2000), ER (Wuhan Servicebio Technology, GB14080, 1:400) and PR (Wuhan Servicebio Technology, GB14137, 1:400). Appropriate conjugated secondary antibodies (Wuhan Servicebio Technology CO. LTD, Wuhan, China) were incubated with the tissue samples for 50 min at room temperature. Quantitative analysis of immunohistochemical staining was performed using ImageJ (National Institutes of Health, Bethesda, MD, USA) and the quantification was calculated as the mean density for each protein (IOD/area). Images were assessed by three investigators who were blinded to each other.

Immunofluorescence Staining

The expression of CD146 and PDGF-Rβ was evaluated by immunofluorescence. The paraffin-embedded sections were deparaffinized in water. Antigen retrieval was performed by immersing sections in EDTA Antigen Retrieval Buffer (pH 8.0) in a microwave oven for 15 min. The sections were incubated with 3% H_2O_2 for 25 minutes at room temperature to eliminate endogenous peroxidase activity, and then blocked with 3% BSA for 30 min at room temperature. Sections were incubated with primary antibodies (CD146, Abcam, ab75769; 1:100) and PDGF-Rβ (Abcam, ab69506; 1:100) overnight at 4°C, followed by incubation with fluorescently-labeled secondary antibodies for 50 minutes at room temperature. DAPI was used to counterstain cell nuclei. Slides were then mounted on a coverslip. Images were obtained using fluorescent microscopy.

Statistical Analysis

Statistical analysis was performed using JMP Pro 16 software (SAS Institute, Inc.) to compare variables. Results are expressed as mean values ± standard deviation. Nonparametric comparisons for each pair were performed using the Wilcoxon Method. P values < 0.05 were considered significant.

Results

Endometrium Morphology During the Implantation Window in Different Age Groups

Histopathological examination of paraffin sections stained with hematoxylin-eosin (Figure 1) revealed characteristics of the secretory phase endometrium: the uterine glands were very coiled with wide lumens in premenopausal women (early and intermediate age groups). However, subjects over the age of 50 years (almost postmenopausal women) were noted to have atrophy of the uterine glands.

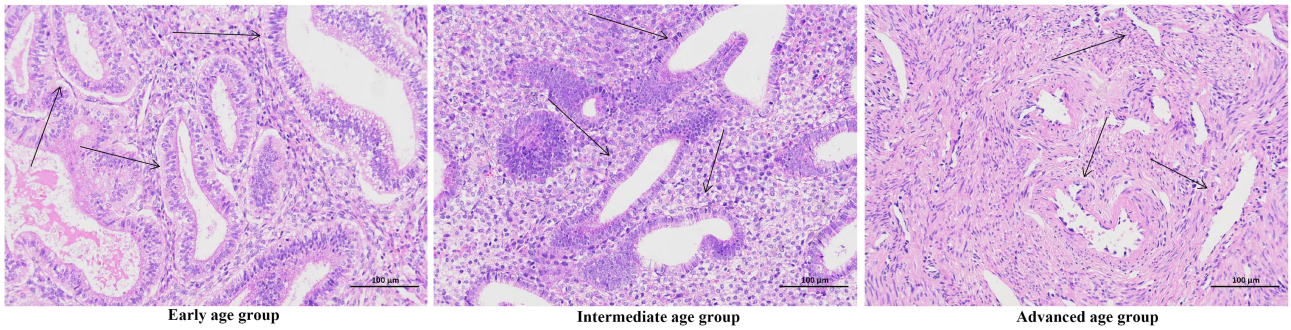

Figure 1 Hematoxylin and eosin staining in human endometrium. The uterine histology detected by Hematoxylin and eosin staining. The uterine glands were very coiled with wide lumens in premenopausal women. However, subjects over the age of 50 years (almost postmenopausal women) were noted to have atrophy of the uterine glands. The arrows indicate the uterine glands.

Endometrial Receptivity Markers and Steroid Hormone Receptor Expression During the Implantation Window in Different Age Groups

As shown in Figure 2, no significant differences in expression of HOXA10 and OPN (p>0.05) were found among the three groups. However, we found a significant difference in LIF expression between the early and advanced age groups, with higher expression noted in the latter group (p=0.02). Similarly, estrogen receptor (ER) and progesterone receptor (PR) expression were significantly increased (p=0.01 and p=0.01, respectively) in the advanced age group compared with the early age group.

Characterization of CD146+ and PDGF-Rβ+ Cells in the Endometrium at Different Ages

CD146 and PDGF-Rβ are not only markers of endometrial stem cells, but also markers of perivascular cells.[16] Therefore, they are mainly expressed in endometrial basalis and functionalis vessels (Figure 3).

In the present study, we tested the positivity, mean density and area density of the combination of CD146 and PDGF-Rβ immunofluorescence staining. No significant differences were found among the three groups (p>0.05).

Discussion

In human reproduction, there is a clear age-related decline in fertility.[4] Therefore, it is essential to delineate the reasons for this decline, so targeted measures may be implemented to improve female fertility. There is little doubt that oocyte quality declines with age,[8] contributing to age-related fertility decline (ARFD). However, as one of the key factors of embryo implantation, the issue of whether endometrial receptivity also declines is still somewhat controversial.[9] In this study, we found that there were no differences in HOXA10 and OPN expression among the three groups. This may support the assumption that endometrial receptivity does not decline with age.

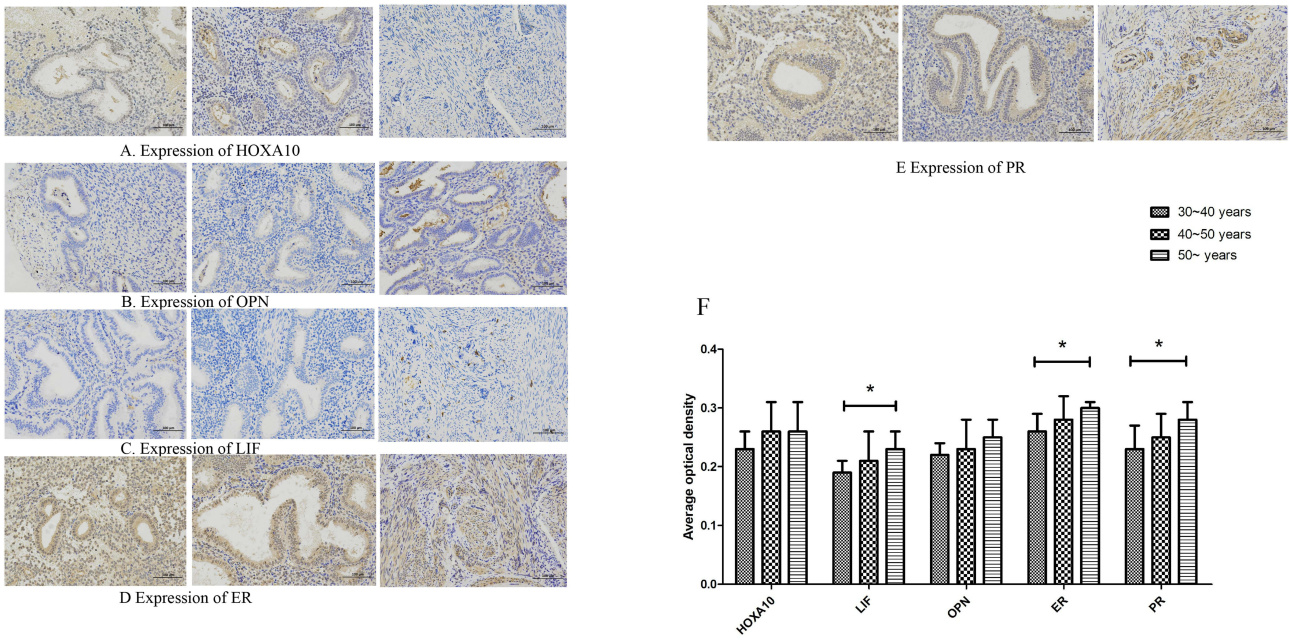

Figure 2 Immunohistochemical staining and semi-quantitative analysis of HOXA, OPN, LIF, ER and PR. No significant differences in expression of HOXA10 and OPN (p>0.05) were found among the three groups. However, we found a significant difference in LIF expression between the early and advanced age groups, with higher expression noted in the latter group (p=0.02). Similarly, estrogen receptor (ER) and progesterone receptor (PR) expression were significantly increased (p=0.01 and p=0.01, respectively) in the advanced age group compared with the early age group. *Means significant difference (P<0.05). (**A-E**) Immunohistochemical staining of HOXA10, OPN, LIF, ER and PR. (**F**) Semi-quantitative analysis of HOXA10, OPN, LIF, ER and PR.

Abbreviations: HOXA10, Homeobox A10; OPN, Osteopontin; LIF, Leukemia inhibitory factor; ER, Estrogen receptor; PR, Progesterone receptor.

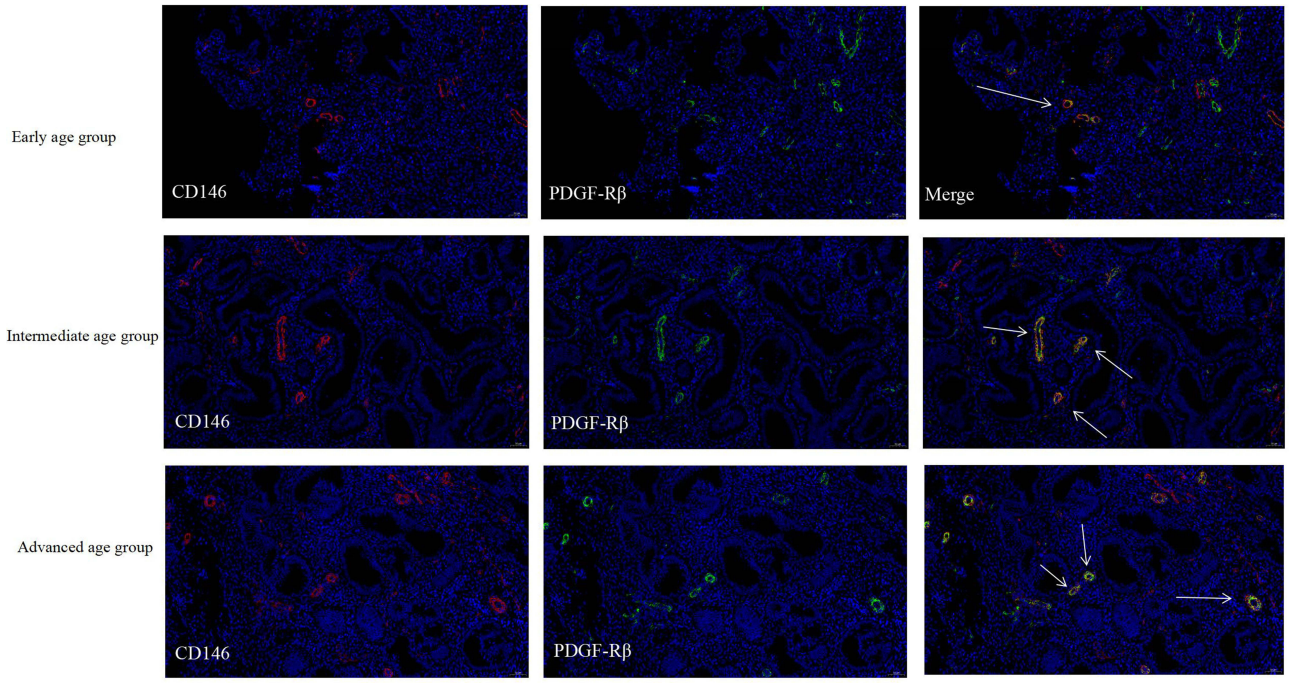

Figure 3 Immunofluorescence staining of CD146+ and PDGF-Rβ+ in human endometrium. For the positivity, mean density and area density of the combination of CD146 and PDGF-Rβ immunofluorescence staining, no significant differences were found among the three groups ($p>0.05$). CD146+ and PDGF-Rβ+ cells are mainly expressed in endometrial basalis and functionalis vessels (arrow).

Similar results were found by Paulson et al. To exclude the potential impact of oocyte quality on fertility, they retrospectively analyzed pregnancy outcomes in patients undergoing oocyte donation. They found that there were no significant differences in overall clinical and cumulative pregnancy rates between different age groups. They concluded that endometrial receptivity remains unaltered with increasing age.[17] One limitation of their study was that pregnancy outcomes do not completely represent endometrial receptivity. Embryo implantation, one of the earliest events in reproduction, happens during a surprisingly short time, however, pregnancy is a complicated process affected by multiple factors. So study of the endometrium during the implantation window is particularly important. Furthermore, Barrenetxea et al also found that there was no relationship between age and the result of an endometrial receptivity assay (ERA) test by multivariable logistic (binomial) analysis.[18]

However, there are also different findings in the literature. Fogle et al found a statistically significant inverse correlation between HOXA10 gene expression in endometrial samples and patient age. This suggests that increasing age negatively impacts embryo implantation at a molecular level.[19] A. Devesa-Peiro et al performed transcriptomic profiling of endometrial samples according to age using a genome-wide functional non-targeted approach. Specifically, there were 5778 differentially expressed genes and 27 significantly altered endometrial functions associated with endometrial gene expression changes related to age. They postulated that endometrial aging may be a critical cause of infertility.[20] These data show different findings at both the clinical and molecular level. Thus, the relationship between advancing age and endometrial receptivity seems to be complex and worthy of further study and exploration.

Serum progesterone and estradiol levels gradually decrease as women age[21] because of hypo-function of the ovary. However, few studies have examined ER and PR expression in the endometrium at different ages. There are only reports on ER and PR changes in accordance with the menstrual cycle.[22] Our research found that expression of ER and PR in women over 50 years old were significantly higher than those in women between the ages of 30 and 40. We speculate that decreased hormone levels may result in a compensatory increase in receptor expression in the endometrium during or after menopause.

Another interesting observation is that even though LIF is also an endometrial receptivity marker, compared with HOXA10 and OPN, its expression differed. In this study, we found that LIF expression was significantly higher in the

advanced age group than in the early age group. LIF receptors are present in the endometrium as well as in placental trophoblasts,[23] which play an important role in embryo implantation as demonstrated in LIF -/- female mice, which undergo implantation failure.[24] LIF regulate endometrial receptivity through taking part in the LIF signaling pathways, such as abnormal LIF/STAT3 signaling can result in implantation failure.[25] Moreover, LIF expression is regulated by cytokines, leptin and estrogen in endometrial cells in humans and rodents.[26] It is thus clear that the changes of LIF expression are the combined effect of several factors. In addition, expression characteristics of different endometrial receptivity markers have not been systematically studied so far, worthing our further investigating and discussion. That we can choose specific marker in different diseases according to their characteristic.

Endometrial mesenchymal stem cells residing in the basal and functional layer of the endometrium, are critical for endometrial regeneration and uterine function. Meixian et al found that umbilical cord mesenchymal stem cells (UCMSCs) expressing HOXA10 could improve endometrial receptivity in a rat endometrial injury model.[27] In addition, another study demonstrated that exosomes isolated from bone marrow-derived stem cells (BMSCs) can facilitate injured endometrial tissue to recover and improve endometrial receptivity.[28] These findings suggest that endometrial stem cells might play a vital role in embryo implantation. Therefore, by studying the source of endometrial progenitor cell populations, we can get a better idea of endometrial physiology, including embryo implantation. However, in our study, we found that expression levels of CD146 and PDGF-Rβ, and by extension endometrial stem cells, do not alter with increasing age.

One strength of this study was our investigation of endometrial receptivity in women aged 50 or older. In reality, these women may benefit greatly from assisted reproductive technologies (ART) and oocyte/embryo donation, such as their only child passes away. There was one report of a 66-year-old woman who underwent in vitro fertilization using donated embryos and gave birth to healthy twin boys.[29] It is telling that, "aging of the endometrium" is not the system's bottleneck. With the use of hormone replacement therapy, it is possible to restore uterine function in postmenopausal women. This also supports the notion that age-related decline in female fecundity is not attributable to reduced endometrial receptivity. Our results further prove this. Nevertheless, we still need to consider ethical issues for older women giving birth. There are also some limitations of this study. First, semi-quantitative analysis of endometrial receptivity markers by immunohistochemistry was performed. Second, we did not analyze reproductive outcome, because advanced age subjects in our study were not desirous of pregnancy. Third, because this was simply a preliminary study, we just investigated the correlation between age and endometrial receptivity. Endometrial receptivity is a result influenced by factors in many aspects, so further studies are needed.

Conclusion

In conclusion, we studied the change of endometrial receptivity markers and endometrial mesenchymal stem cell surface markers in women of different ages during embryo implantation period. By analyzed with immunohistochemistry in advanced age group, the expression of LIFR, ER and PR in endometrium are higher than those in younger patients, however, no differences were found between the groups for other endometrial receptivity markers (HOXA10, OPN), they could have different effects on endometrial receptivity. Furthermore, we found no correlation between eMSC surface markers expression and age, While this is only a preliminary study, there must be many point needed further improving in this thesis, especially on the aspect of quantitative analysis of endometrial receptivity markers need further deepen and detail, such as their RNA and protein expression. In addition the interaction mechanism needed to be researched deeply.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to take responsibility and be accountable for the contents of the article.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Vander BM, Christine W. Fertility and infertility: definition and epidemiology. *Clin Biochem*. 2018;62:2–10. doi:10.1016/j.clinbiochem.2018.03.012
2. Renu B, Vertika S, Singh R, Kiran S. Environment, Lifestyle, and Female Infertility. *Reprod Sci*. 2021;28(3):617–638. doi:10.1007/s43032-020-00279-3
3. Wood JW. Fecundity and natural fertility in humans. *Oxf Rev Reprod Biol*. 1989;11:61–109.
4. American College of Obstetricians and Gynecologists Committee on Gynecologic Practice and Practice Committee. Female age-related fertility decline. Committee Opinion No. 589. *Fertil Steril*. 2014;101(3):633–634. doi:10.1016/j.fertnstert.2013.12.032
5. Eftekhari MAR, Taheri MM, Masoud H, Ghasem S. Oocyte quality and aging. *JBRA Assist Reprod*. 2022;26(1):105–122. doi:10.5935/1518-0557.20210026
6. Jinyan Z, Qing C, Xiang X. An update on the progress of endometrial receptivity in women with polycystic ovary syndrome. *Reprod Sci*. 2022;29(8):2136–2144. doi:10.1007/s43032-021-00641-z
7. Paulson RJ. Introduction: endometrial receptivity: evaluation, induction and inhibition. *Fertil Steril*. 2019;111(4):609–610. doi:10.1016/j.fertnstert.2019.02.029
8. Rotsztejn DA, Ord T, Balmaceda JP, Asch RH. Variables which influence the selection of an egg donor. *Hum Reprod*. 1992;7(1):59–62. doi:10.1093/oxfordjournals.humrep.a137558
9. Meldrum DR. Female reproductive aging--ovarian and uterine factors. *Fertil Steril*. 1993;59(1):1–5. doi:10.1016/s0015-0282(16)55608-8
10. Shaghayegh B, Marion RJ, Merli S, Marie TL, Andres S, Alireza F. Potential innate immunity-related markers of endometrial receptivity and recurrent implantation failure (RIF). *Reprod Biol*. 2021;21(4):100569. doi:10.1016/j.repbio.2021.100569
11. Gemma C, Jaume O, Montserrat C, et al. Osteopontin and alphavbeta3 integrin as markers of endometrial receptivity: the effect of different hormone therapies. *Reprod Biomed Online*. 2010;21(3):349–359. doi:10.1016/j.rbmo.2010.04.012
12. Lessey BA. The use of integrins for the assessment of uterine receptivity. *Fertil Steril*. 1994;61(5):812–814. doi:10.1016/s0015-0282(16)56688-6
13. Mercedes MM, Virginia L, Jorgelina V. Aberrant Hoxa10 gene methylation as a mechanism for endosulfan-induced implantation failures in rats. *Mol Cell Endocrinol*. 2022;547:111576. doi:10.1016/j.mce.2022.111576
14. Parasar P, Sacha CR, Ng N, et al. Differentiating mouse embryonic stem cells express markers of human endometrium. *Reprod Biol Endocrinol*. 2017;15(1):52. doi:10.1186/s12958-017-0273-2
15. Zhongxun L, Guijun Y, Qiang D, et al. Transplantation of human endometrial perivascular cells with elevated CYR61 expression induces angiogenesis and promotes repair of a full-thickness uterine injury in rat. *Stem Cell Res Ther*. 2019;10(1):179. doi:10.1186/s13287-019-1272-3
16. Dominici M, Le BK, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8(4):315–317. doi:10.1080/14653240600855905
17. Paulson RJ, Hatch IE, Lobo RA, Sauer MV. Cumulative conception and live birth rates after oocyte donation: implications regarding endometrial receptivity. *Hum Reprod*. 1997;12(4):835–839. doi:10.1093/humrep/12.4.835
18. Barrenetxea G, Romero I, Celis R, Abio A, Bilbao M, Barrenetxea J. Correlation between plasmatic progesterone, endometrial receptivity genetic assay and implantation rates in frozen-thawed transferred euploid embryos. A multivariate analysis. *Eur J Obstet Gynecol Reprod Biol*. 2021;263:192–197. doi:10.1016/j.ejogrb.2021.05.047
19. Fogle Robin H, Aimin L, Paulson Richard J. Modulation of HOXA10 and other markers of endometrial receptivity by age and human chorionic gonadotropin in an endometrial explant model. *Fertil Steril*. 2010;93(4):1255–1259. doi:10.1016/j.fertnstert.2008.11.002
20. Devesa PA, Sebastian LP, Parraga LA, Pellicer A, Diaz GP. Breaking the ageing paradigm in endometrium: endometrial gene expression related to cilia and ageing hallmarks in women over 35 years. *Hum Reprod*. 2022;37(4):762–776. doi:10.1093/humrep/deac010
21. Rong L, Yan S, Jiawen L, et al. Sex hormone levels in females of different ages suffering from depression. *BMC Womens Health*. 2021;21(1):215. doi:10.1186/s12905-021-01350-0
22. Cao ZY, Eppenberger U, Roos W, Torhorst J, Almendral A. Cytosol estrogen and progesterone receptor levels measured in normal and pathological tissue of endometrium, endocervical mucosa and cervical vaginal portion. *Arch Gynecol*. 1983;233(2):109–119. doi:10.1007/BF02114787
23. Nicola Nicos A, Babon Jeffrey J. Leukemia inhibitory factor (LIF). *Cytokine Growth Factor Rev*. 2015;26(5):533–544. doi:10.1016/j.cytogfr.2015.07.001
24. Stewart CL, Kaspar P, Brunet LJ, et al. Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature*. 1992;359(6390):76–79. doi:10.1038/359076a0
25. Ling S, Yingying Z, Shiqiao P, Xinyi W, Zhongyan S, Weiping T. Implantation failure in rats with subclinical hypothyroidism is associated with LIF/STAT3 signaling. *Endocr Connect*. 2019;8(6):718–727. doi:10.1530/EC-19-0185
26. Inkyu Y, Soogil C, Jisoo H, Soohyung L, Jong KH, Hakhyun K. Leukemia inhibitory factor and its receptor: expression and regulation in the porcine endometrium throughout the estrous cycle and pregnancy. *Asian Australas J Anim Sci*. 2019;32(2):192–200. doi:10.5713/ajas.18.0429
27. Meixian W, Yuanyuan L, Yiwei W, et al. HOXA10 expressing UCMSCs transplantation improved endometrial receptivity on endometrial injury. *Curr Stem Cell Res Ther*. 2022. doi:10.2174/1574888X17666220919111814
28. Qianqian Z, Shengluan T, Yanwen Z, Chen D, Jialyu H, Jiaying L. Exosomes derived from CTF1-modified bone marrow stem cells promote endometrial regeneration and restore fertility. *Front Bioeng Biotechnol*. 2022;10:868734. doi:10.3389/fbioe.2022.868734
29. Simó GM, Calaf AJ, Terribas SN, Luqui SN, Plana BJ, Polo RA. Pregnancy beyond 65: report of a unique case and discussion of a controversial issue. *Eur J Contracept Reprod Health Care*. 2016;21(6):496–498. doi:10.1080/13625187.2016.1234599