Clinical and Experimental Obstetrics & Gynecology

Clin. Exp. Obstet. Gynecol. **2024**; 51(1): 4
https://doi.org/10.31083/j.ceog5101004

Original Research

Decreased Expression of Cav-1 Is Associated with Compromised Endometrial Angiogenesis during the Implantation Window in PCOS Rats: A Prospective Randomized Laboratory-Based Study

Huayun Xu[1], Guozeng Wang[2,*]

[1]Department of Traditional Chinese Medicine, Shanghai General Hospital, School of Medicine, Shanghai Jiao Tong University, 200080 Shanghai, China
[2]Department of Obstetrics and Gynecology, Shanghai East Hospital, School of Medicine, Tongji University, 200120 Shanghai, China
*Correspondence: zengerwong@126.com (Guozeng Wang)

Academic Editor: Michael H. Dahan
Submitted: 25 May 2023 Revised: 15 August 2023 Accepted: 22 August 2023 Published: 8 January 2024

Abstract

Background: Caveolin-1 (Cav-1) is known to regulate angiogenesis. However, little is known about Cav-1's role in polycystic ovary syndrome (PCOS). This study aims to investigate Cav-1's expression in the endometrium of PCOS rats during the implantation window and its association with endometrial angiogenesis. **Methods**: Female Sprague Dawley (SD) rats were randomly divided into the control and PCOS groups. The rats in the PCOS group mated after ovulation induction, while the rats in the control group mated during the estrus period. On the 2nd and 5th days of pregnancy, the rats were sacrificed, and the endometrium was isolated from their uteruses. Immunohistochemistry (IHC) staining of CD34 was used to evaluate the endometrial micro-vessel density (MVD). The expression of Cav-1 and vascular endothelial growth factor (VEGF) in the endometrium of both groups was assessed through IHC staining and real-time reverse transcription polymerase chain reaction (RT-PCR) analysis. **Results**: IHC analysis of endometrium tissue sections showed reduced MVD in PCOS rats on both the 2nd and 5th days of pregnancy. The endometrial expression of Cav-1 and VEGF were also significantly downregulated in the PCOS group compared to the control group during the implantation window. Interestingly, the endometrial expression of Cav-1 was positively correlated with MVD and VEGF. **Conclusions**: Our study demonstrated the decreased endometrial angiogenesis in PCOS rats during implantation window. This decrease was linked to decreased Cav-1 expression, suggesting Cav-1 is a potential therapeutic target for PCOS patients.

Keywords: Cav-1; endometrial angiogenesis; endometrial receptivity; polycystic ovarian syndrome

1. Introduction

Polycystic ovary syndrome (PCOS) is a dysovulation disorder that affects female reproductive endocrine and metabolic dysfunction, leading to infertility in women [1]. While methods to promote follicle maturation and induce ovulation can improve ovulation rates, PCOS patients still face challenges with low pregnancy and high abortion rates [2,3]. Previous research has identified that PCOS patients experience issues with endometrial development and embryo receptivity, which contributes to infertility following ovulation promotion [4]. Good endometrial blood perfusion is an important condition for the establishment of endometrial receptivity. Insulin resistance [5,6], high luteinizing hormone, and high androgen [7,8] can lead to insufficient uterine blood perfusion in PCOS patients, which negatively impacts endometrial receptivity [9].

Caveolin-1 (Cav-1) is a surface marker protein of plasma membrane vesicles. It is abundantly expressed in endothelial cells, participates in cell proliferation and differentiation, tissue angiogenesis, and signal transduction, and is closely related to angiogenesis [10]. At present, it has been widely reported that Cav-1 regulated tumor growth and angiogenesis following cerebral ischemia, however, its role in endometrial receptivity remains unstudied. This study aims to investigate the Cav-1 expression in the endometrium of PCOS rats during the implantation window period and examine its correlation relationship with endometrial angiogenesis, to discover potential therapeutic targets for improving the endometrial receptivity of PCOS patients.

2. Materials and Methods

2.1 Experimental Animals

Twelve virgin female Sprague Dawley (SD) rats weighing about 50 ± 10 g were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., at the age of three weeks. Animals were housed in standard animal houses, and all animal experiments complied with Animal Research: Reporting of *in vivo* Experiments (ARRIVE) guidelines and were carried out following the Ethical Committee guidelines on the use of experimental animals at Shanghai Jiao Tong University, School of Medicine.

2.2 Construction of PCOS Model and Rat Mating

Rats were fed adaptively for three days and randomly divided into the PCOS group (n = 6) and the control group (n

= 6). The PCOS group was fed a high-fat pellet feed (Wuxi Fan Bo Biotechnology Co., Ltd, Wuxi, Jinagsu, China). According to Lee MT *et al*. [11], dehydroepiandrosterone (DHEA, 60 mg/kg) (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 0.2 mL oil injection (Sigma-Aldrich, St. Louis, MO, USA). The rats in the control group were subcutaneously injected with 0.2 mL oil injection on the back of the neck every day, and those in the PCOS group were subcutaneously injected with 0.2 mL DHEA + oil injection on the back of the neck every day for 20 days. Vaginal smears were taken from the 10th day of injection to observe the two estrous cycles. The continuous appearance of keratinocytes indicated the successful establishment of the PCOS model. Afterward, the PCOS group received 0.52 mg/kg letrozole (Jiangsu Hengrui Pharmaceutical Co., LTD, Lianyungang, Jiangsu, China) by gavage, while the control group received 2.1 mL/kg normal saline by gavage. At 04:00 PM on the 2nd day after gavage, female and male SD rats in both groups mated during estrus in a ratio of 1:1. The presence of a vaginal plug the next morning indicated pregnancy.

2.3 Tissue Processing

The rats in both groups were sacrificed on the 2nd and 5th days of pregnancy. Under the aseptic condition, the bilateral uteruses were removed and the endometrium was separated by cutting the uterine horn.

2.4 Observation Parameters

2.4.1 Estrous Cycle and Weight Change

Vaginal smears of rats were obtained and fixed every morning beginning on the 10th day of oil injection, and the changes in the estrous cycle were then analyzed under a microscope (Olympus Corporation, Tokyo, Japan). Furthermore, the rats' bodyweight was assessed once a week.

2.4.2 Immunohistochemistry for Micro-Vessel Density (MVD), Cav-1, and Vascular Endothelial Growth Factor (VEGF)

Endometrial tissue from rats was extracted and fixed in paraffin on the 2nd and 5th day of pregnancy, as described aboved. The following procedures were used for immunohistochemistry (IHC) staining: deparaffinization of tissue slides was followed by antigen retrieval. The slides were incubated with primary antibodies (as mentioned below), followed by horseradish peroxidase (HRP)-conjugated secondary antibodies (as mentioned below), and diaminobenzidine (DAB, Abcam, Fremont, CA, USA) after endogenous peroxidase was blocked with 3% H_2O_2 solution. The slides were then counterstained with hematoxylin (Abcam, Cambridge, CA, USA). The following primary antibodies were used: CD34 (ab198823, Abcam, Cambridge, CA, USA) 1:2500 dilution; vascular endothelial growth factor A (VEGFA) (ab1316, Abcam, Cambridge, CA, USA) 1:250 dilution; Caveolin-1 (ab32577, Abcam, Cambridge, CA, USA) 1:2000 dilution. The secondary antibodies were: Biotinylated goat-anti-rabbit IgG (Beyotime, A0208, Shanghai, China) 1:50 dilution; Biotinylated goat-anti-mouse IgG (Beyotime, A0216) 1:50 dilution. The micro-vessel density (MVD) of immunohistochemical endometrial sections was determined using the standard of Weidner *et al*. [12].

2.4.3 Real-Time RT-PCR Analysis of Cav-1 and VEGF Expression

The endometrium of the 5th day of pregnancy was harvested and homogenized, and total RNA was extracted suing Trizol (Invitrogen, Carlsbad, CA, USA). Then, 1 µg of extracted total RNA was reverse transcribed using the Reverse transcription kit following the manufacturer's instructions (Ambion Company, Austin, TX, USA), and 10 ng cDNA was used for PCR amplification (PrimeScript™ RT reagent Kit, TAKARA, Kusatsu, Shiga, Japan), and mRNA expression was quantified using the $2^{-\Delta\Delta ct}$ method. The primer sequences are listed in Table 1.

Table 1. The oligonucleotide sequences of primers used for RT-PCR.

Primer name	Sequence (5′→3′)
β-actin-F	TCGTACCACTGGCATTGTGAT
β-actin-R	AGGGAGCGCGTAACCC
Cav-1-F	CAACGACGACGTGGTCAAGA
Cav-1-R	CATAGGGATGCCGAAGATGGT
VEGF-F	CGACAGAAGGGGAGCAGAAAG
VEGF-R	AATTGGACGGCAATAGCTGC

Cav-1, Caveolin-1; VEGF, vascular endothelial growth factor; F, Forward; R, Reverse; RT-PCR, real-time reverse transcription polymerase chain reaction.

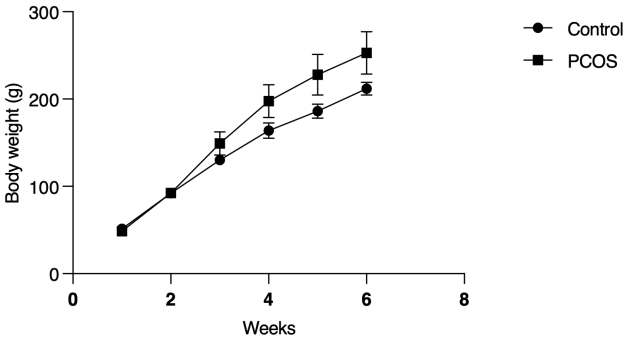

Fig. 1. Bodyweight growth trend of the two groups of rats. In the PCOS group, the bodyweight increased significantly from the 4th week of high-fat feeding, and it was more evident than that in the control group at the 6th week. PCOS, polycystic ovary syndrome.

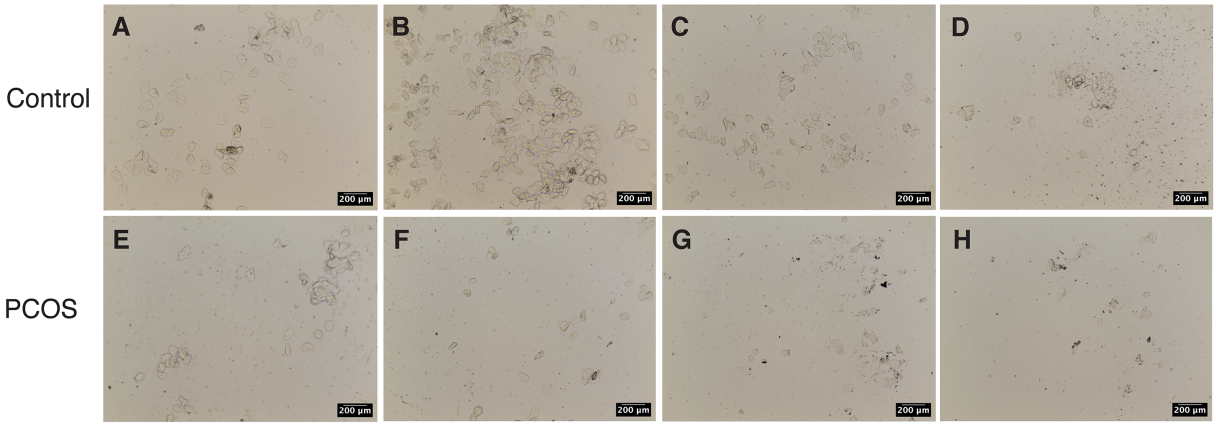

Fig. 2. Vaginal smears. (A–D) A normal estrous cycle exists in the control group. (A) Proestrus. (B) Estrous period. (C) Metaestrus. (D) Estrus interval. (E–H) When compared with the control group, the rats in the PCOS group were always in the estrus interval and lost the regular estrous cycle after building.

2.5 Statistical Analysis

Statistical analysis was performed with SPSS (version 20.0; IBM, Armonk, NY, USA). All data followed normal distributions and were presented as mean ± standard deviation. The independent *t*-test was used to examine the differences between the two groups. Pearson correlation analysis was used to investigate relationships between Cav-1 and MVD/vascular endothelial growth factor (VEGF). A probability value of less than 0.05 was deemed statistically significant.

3. Results

3.1 Increased Bodyweight and Arrested Estrous Cycle in PCOS Group Rats

Starting in the fourth week, high-fat feeding induced significant weight gain in the PCOS group rats (Fig. 1). We also observed that rats in the PCOS group exhibited irregular estrous cycles during the estrus period, whereas rats in the control group had regular cycles, indicating the successful establishment of PCOS model (Fig. 2).

3.2 Decreased MVD in PCOS Rats during Implantation Window

To evaluate the endometrial blood perfusion during pregnancy, we immunoassayed the endometrium slices with CD34, a microvessel-specific marker. As shown in the figure, on the 2nd and 5th days of pregnancy, the MVD in the PCOS group's endometrium was significantly lower than in the control group. Furthermore, MVD increased with gestation in both control and PCOS groups, although there was no statistical significance (Fig. 3A–D).

3.3 Downregulated Expression of Endometrial Cav-1 and VEGF in PCOS Rats during Implantation Window

Cav-1 and VEGF protein were mainly expressed in endometrial stromal and glandular epithelial cells [13,14]. We utilized immunohistochemical labeling on endometrial slices to investigate the alterations in Cav-1 and VEGF expression in PCOS rat models. Our data revealed that the expression of Cav-1 and VEGF protein in the endometrium of the PCOS group was significantly lower than that of the control group during the implantation window stage. In addition, we observed slightly upregulated expression of VEGF in the PCOS group on the 5th day compared to the 2nd day. In both the control and PCOS groups, the expression of Cav-1 increased over time. However, no statistical significance was seen (Fig. 3E–L). On the 5th day of pregnancy, real-time reverse transcription polymerase chain reaction (RT-PCR) analysis revealed that the expression of Cav-1 and VEGF mRNA in the endometrium of the PCOS group was significantly lower than that of the control group (Fig. 4).

3.4 Endometrial Cav-1 Expression is Associated with MVD and VEGF Expression

We further explored the association between Cav-1 and endometrial angiogenesis. Interestingly, correlation analysis showed that Cav-1 expression was positively correlated with MVD (r = 0.818, p = 0.047) and VEGF (r = 0.835, p = 0.039) in the endometrium of the PCOS group at the implantation window stage (Fig. 5), suggesting that Cav-1 regulates endometrial angiogenesis.

4. Discussion

PCOS is a prevalent metabolic and ovulatory disorder in women of childbearing age, with a prevalence rate as high as 4–15% [15]. According to research, in addition to ovulation abnormalities, poor endometrial receptivity is an important cause of PCOS infertility [16,17]. Endometrial receptivity is the ability of the endometrium to facilitate embryo implantation during a specified period [18], which necessitates appropriate endometrial blood vessel develop-

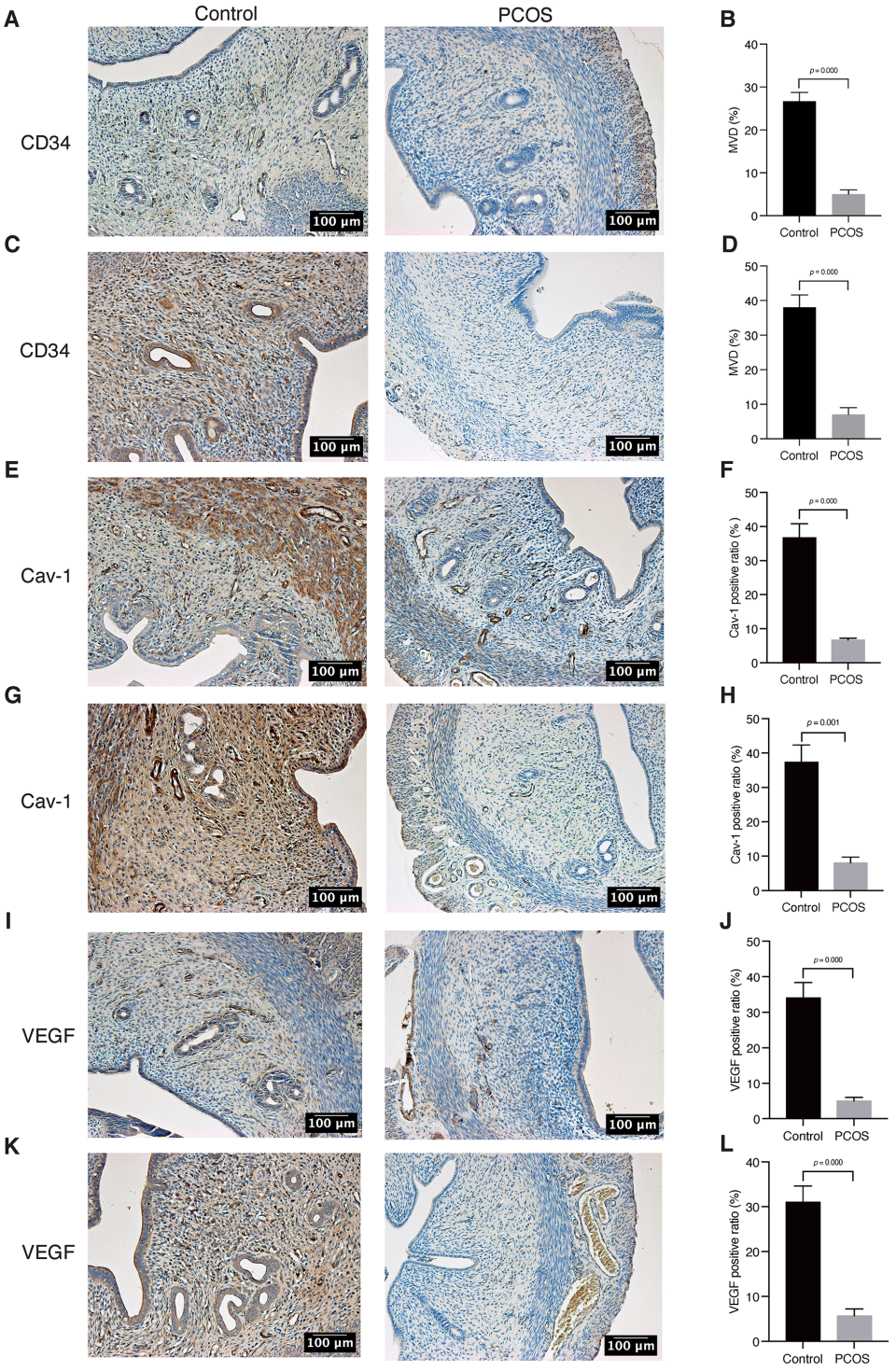

Fig. 3. Tissues CD34, Cav-1, and VEGF expression by IHC. (A,B) On the 2nd day of pregnancy, IHC staining for the expression of CD34. (C,D) On the 5th day of pregnancy, IHC staining for the expression of CD34. (E,F) On the 2nd day of pregnancy, IHC staining for the expression of Cav-1. (G,H) On the 5th day of pregnancy, IHC staining for the expression of Cav-1. (I,J) On the 2nd day of pregnancy, IHC staining for the expression of VEGF. (K,L) On the 5th day of pregnancy, IHC staining for the expression of VEGF. Original magnification: 200×. IHC, Immunohistochemistry. MVD, micro-vessel density.

ment. It is also a prerequisite for embryo implantation and pregnancy to proceed successfully [19,20].

MVD is a crucial parameter for assessing angiogenesis. A greater extent of neovascularization is indicated by a higher MVD in tissues. Endometrial blood flow may there-

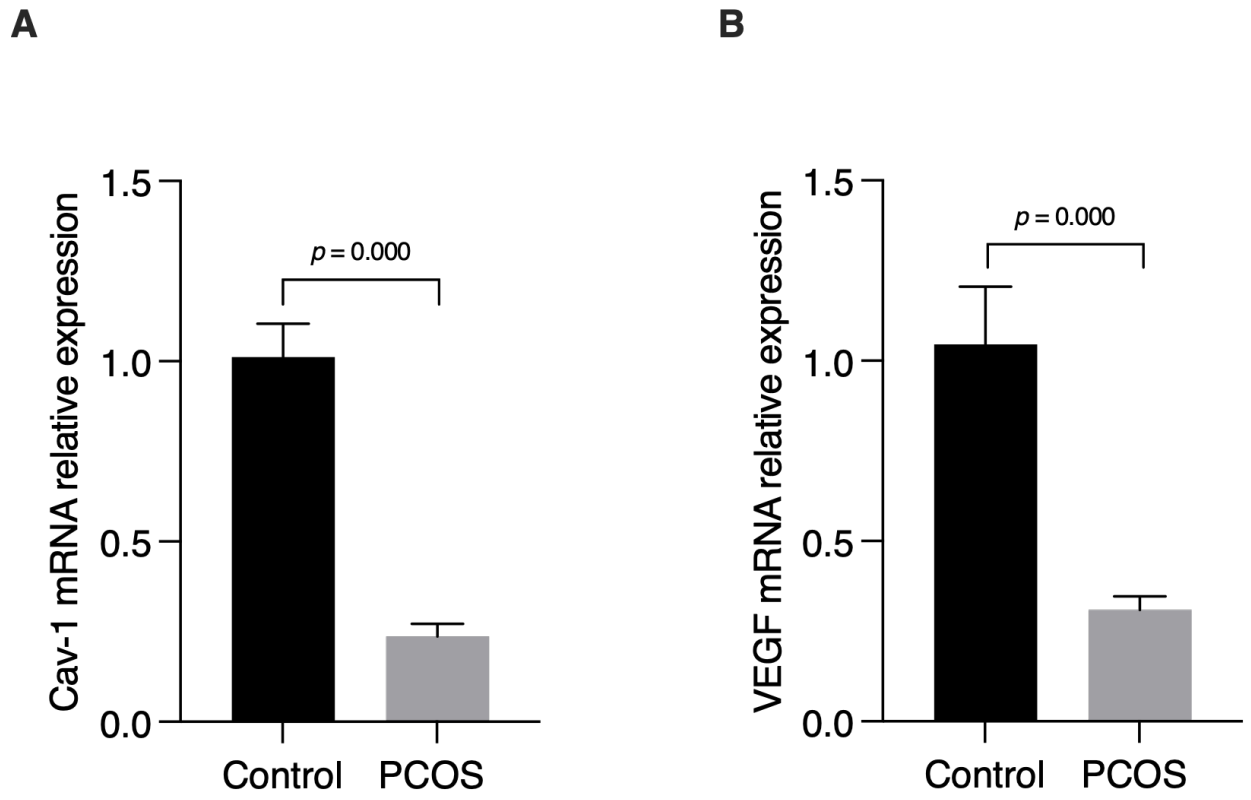

Fig. 4. Tissues Cav-1 and VEGF mRNA expression by real-time reverse transcription polymerase chain reaction (RT-PCR). (A,B) On the 5th day of pregnancy, the expression of Cav-1 and VEGF mRNA in the endometrium of the PCOS group was significantly lower than that in the control group. A significant difference was noted between the PCOS and control groups.

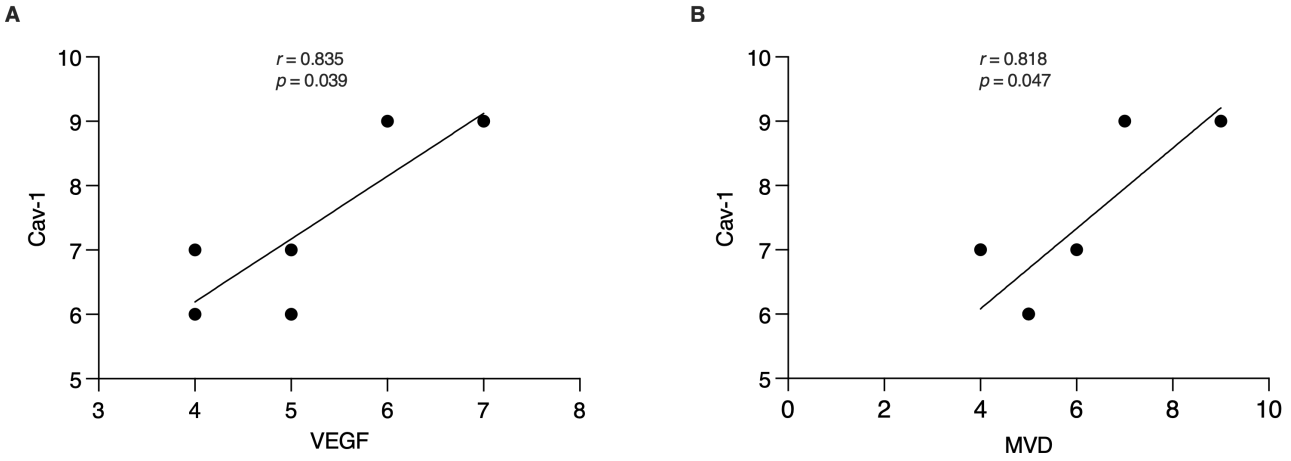

Fig. 5. Association between Cav-1 and endometrial angiogenesis. (A,B) According to the correlational analysis, in the PCOS group, Cav-1 was positively correlated with MVD and the VEGF expression. The coefficient of determination of Cav-1 and VEGF was r = 0.835, p = 0.039. The coefficient of determination of Cav-1 and MVD was r = 0.818, p = 0.047.

fore be well determined by assessing endometrial MVD by immunostaining endometrial microvascular endothelial cells with CD34 antibody [21]. Our findings revealed an increase in MVD as gestational days progressed (Fig. 3A–D). During the implantation window time, however, the MVD of the endometrium in the PCOS group was significantly lower than in the control group (Fig. 3A–D). This suggests that endometrial angiogenesis was decreased, resulting in insufficient blood flow during the implantation window in PCOS individuals.

VEGF is an important angiogenesis-promoting cytokine. According to research, VEGF primarily affects endothelial cells in endometrial vessels, stimulating their proliferation and migration [22,23]. Because VEGF expres-

sion closely correlates with uterine blood flow, it is a significant marker of endometrial receptivity [24,25]. Inadequate VEGF levels have been linked to decreased endometrial receptivity, which may potentially impact the development of early villi [26]. Consequently, VEGF plays an important role in endometrial neovascularization [27]. Our findings show that VEGF expression is lower in the endometrium of PCOS rats during the implantation window period (Fig. 3I–L), aligning with the lower levels of endometrial MVD in those rats. These results reinforce the notion that endometrium angiogenesis is impaired during the PCOS implantation window, potentially affecting endometrial receptivity, development, and embryo implantation. However, our data also reveal that VEGF expression is downregulated in the conrol group during gestation, which contradicts the MVD results. Given the small sample size of rats in this experiment, this discrepancy may be due to experimental errors.

Caveolae are vesicle-like structures formed by the invagination of the cytoplasmic membrane [28]. Among these structures, Cav-1 is a crucial structural protein [28] that is highly expressed in vascular endothelial cells [29]. It has been found that Cav-1 participates in the process of angiogenesis and is a key regulator of this process [30]. As a result, it has garnered significant attention in tumor and cerebral ischemia research. Given that the biological behavior of embryo implantation resembles that of tumor invasion and metastasis, the successful implantation of an embryo relies on a well-supplied endometrium. Therefore, Cav-1 may become a new target for investigating endometrial angiogenesis. In this study, we found a significant reduction in Cav-1 expression in the endometrium of the PCOS group compared to the control group (Fig. 3E–H). Furthermore, this reduction was positively correlated with the expression of MVD and VEGF (Fig. 5). Recent study has also confirmed that Cav-1 can influence VEGF expression in umbilical vascular endothelial progenitor cells [31]. After Cav-1 gene knockout, the number of VEGF-mediated blood vessels decreased significantly [32]. Collectively, these findings suggest that the decreased expression of Cav-1 in the endometrium during the PCOS implantation window may be an important reason for the decrease in endometrial angiogenesis.

According to research, the chronic inflammatory process plays an important role in the pathogenesis of PCOS [33]. Some inflammatory markers, such as tumor necrosis factor-alpha (TNF-α) [34], nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [35] levels increased in PCOS patients. Existing studies have found that inflammatory factors play an important role in promoting angiogenesis in hypoxic tumors and acute and chronic inflammation [36,37], that low TNF-α levels can induce angiogenesis in a variety of tumors [38], and that NF-κB can promote angiogenesis and induce disease in various inflammatory areas [37]. Recent studies have shown that obesity is a chronic low-grade inflammatory state. Cav-1 expression is increased in the adipose tissue of obese patients, and it is positively correlated with TNF-α and NF-κB [33,34,39]. However, it is still unclear whether Cav-1 regulates endometrial angiogenesis in the implantation window of PCOS via inflammatory mediators such as TNF-α and NF-κB and their related mechanisms, which requires further investigation in the future.

5. Conclusions

Our study found that PCOS rats have reduced endometrial angiogenesis during the implantation window. This reduction might be attributed to the decreased Cav-1 expression, indicating that Cav-1 could be a potential therapeutic target for PCOS infertility.

Abbreviations

Cav-1, Caveolin-1; PCOS, polycystic ovary syndrome; SD, Sprague Dawley; MVD, micro-vessel density; IHC, immunohistochemistry; VEGF, vascular endothelial growth factor; DHEA, dehydroepiandrosterone; TNF-α, tumor necrosis factor-alpha; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells.

Availability of Data and Materials

Data are available upon reasonable request.

Author Contributions

HYX contributed to study design, data acquisition, data analysis, and manuscript draft. GZW organized the whole study and rewrote the manuscript. Both authors read and approved the final manuscript. Both authors contributed to editorial changes in the manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the Clinical Center Laboratory Animal Welfare & Ethics Committee of Shanghai General Hospital (Ethics approval number: 2021AWS0171).

Acknowledgment

Not applicable.

Funding

This research was supported by the Special Project of Traditional Chinese Medicine Scientific Research of Shanghai Health and Family Planning Commission (2016LP024) and “Xinglin Xinxing” Talent Project of Shanghai [ZY(2018-2020)-RCPY-3006].

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. Lancet (London, England). 2007; 370: 685–697.

[2] Lopes IMRS, Baracat MCP, Simões MDJ, Simões RS, Baracat EC, Soares JM Jr. Endometrium in women with polycystic ovary syndrome during the window of implantation. Revista da Associacao Medica Brasileira (1992). 2011; 57: 702–709.

[3] Jakubowicz DJ, Iuorno MJ, Jakubowicz S, Roberts KA, Nestler JE. Effects of metformin on early pregnancy loss in the polycystic ovary syndrome. The Journal of Clinical Endocrinology and Metabolism. 2002; 87: 524–529.

[4] Schulte MMB, Tsai JH, Moley KH. Obesity and PCOS: the effect of metabolic derangements on endometrial receptivity at the time of implantation. Reproductive Sciences (Thousand Oaks, Calif.). 2015; 22: 6–14.

[5] Ujvari D, Jakson I, Babayeva S, Salamon D, Rethi B, Gidlöf S, *et al*. Dysregulation of In Vitro Decidualization of Human Endometrial Stromal Cells by Insulin via Transcriptional Inhibition of Forkhead Box Protein O1. PLoS ONE. 2017; 12: e0171004.

[6] Li R, Wu J, He J, Wang Y, Liu X, Chen X, *et al*. Mice endometrium receptivity in early pregnancy is impaired by maternal hyperinsulinemia. Molecular Medicine Reports. 2017; 15: 2503–2510.

[7] Lam P, Johnson I, Raine-Fenning N. Endometrial blood flow is impaired in women with polycystic ovarian syndrome who are clinically hyperandrogenic. Ultrasound in Obstetrics & Gynecology. 2009; 34: 326–334.

[8] Ajossa S, Guerriero S, Paoletti AM, Orrù M, Floris S, Mannias M, *et al*. Uterine perfusion and hormonal pattern in patients with polycystic ovary syndrome. Journal of Assisted Reproduction and Genetics. 2001; 18: 436–440.

[9] Wang T, Kang X, Zhao A, He L, Liu Z, Liu F. Low-dose aspirin improves endometrial receptivity in the midluteal phase in unexplained recurrent pregnancy loss. International Journal of Gynaecology and Obstetrics. 2020; 150: 77–82.

[10] Ito A, Shiroto T, Godo S, Saito H, Tanaka S, Ikumi Y, *et al*. Important roles of endothelial caveolin-1 in endothelium-dependent hyperpolarization and ischemic angiogenesis in mice. American Journal of Physiology. Heart and Circulatory Physiology. 2019; 316: H900–H910.

[11] Lee MT, Anderson E, Lee GY. Changes in ovarian morphology and serum hormones in the rat after treatment with dehydroepiandrosterone. The Anatomical Record. 1991; 231: 185–192.

[12] Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis–correlation in invasive breast carcinoma. The New England Journal of Medicine. 1991; 324: 1–8.

[13] Ormazabal P, Romero C, Quest AFG, Vega M. Testosterone modulates the expression of molecules linked to insulin action and glucose uptake in endometrial cells. Hormone and Metabolic Research. 2013; 45: 640–645.

[14] Le AW, Shan L, Wang ZH, Dai XY, Xiao TH, Zuo R. Effects of icariin on the expression of ER, VEGF, and KDR in the endometrial cells of thin endometrium. Genetics and Molecular Research. 2015; 14: 11250–11258.

[15] Fauser BCJM, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, *et al*. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertility and Sterility. 2012; 97: 28–38.e25.

[16] Li SY, Song Z, Song MJ, Qin JW, Zhao ML, Yang ZM. Impaired receptivity and decidualization in DHEA-induced PCOS mice. Scientific Reports. 2016; 6: 38134.

[17] Kamalanathan S, Sahoo JP, Sathyapalan T. Pregnancy in polycystic ovary syndrome. Indian Journal of Endocrinology and Metabolism. 2013; 17: 37–43.

[18] Paulson RJ. Introduction: Endometrial receptivity: evaluation, induction and inhibition. Fertility and Sterility. 2019; 111: 609–610.

[19] Patel BG, Lessey BA. Clinical assessment and management of the endometrium in recurrent early pregnancy loss. Seminars in Reproductive Medicine. 2011; 29: 491–506.

[20] Karizbodagh MP, Rashidi B, Sahebkar A, Masoudifar A, Mirzaei H. Implantation Window and Angiogenesis. Journal of Cellular Biochemistry. 2017; 118: 4141–4151.

[21] Yu X, Gao C, Dai C, Yang F, Deng X. Endometrial injury increases expression of hypoxia-inducible factor and angiogenesis in the endometrium of women with recurrent implantation failure. Reproductive Biomedicine Online. 2019; 38: 761–767.

[22] Nash AD, Baca M, Wright C, Scotney PD. The biology of vascular endothelial growth factor-B (VEGF-B). Pulmonary Pharmacology & Therapeutics. 2006; 19: 61–69.

[23] Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nature Medicine. 2003; 9: 669–676.

[24] Rabbani ML, Rogers PA. Role of vascular endothelial growth factor in endometrial vascular events before implantation in rats. Reproduction (Cambridge, England). 2001; 122: 85–90.

[25] Horner A, Bishop NJ, Bord S, Beeton C, Kelsall AW, Coleman N, *et al*. Immunolocalisation of vascular endothelial growth factor (VEGF) in human neonatal growth plate cartilage. Journal of Anatomy. 1999; 194: 519–524.

[26] Sugino N, Kashida S, Karube-Harada A, Takiguchi S, Kato H. Expression of vascular endothelial growth factor (VEGF) and its receptors in human endometrium throughout the menstrual cycle and in early pregnancy. Reproduction (Cambridge, England). 2002; 123: 379–387.

[27] Zhao DM, Shan YH, Li FH, Jiang L, Qu QL. Correlation between endometrial receptivity with expressions of IL-1 and VEGF in rats with polycystic ovary syndrome. European Review for Medical and Pharmacological Sciences. 2019; 23: 5575–5580.

[28] Enyong EN, Gurley JM, De Ieso ML, Stamer WD, Elliott MH. Caveolar and non-Caveolar Caveolin-1 in ocular homeostasis and disease. Progress in Retinal and Eye Research. 2022; 91: 101094.

[29] Tu F, Pang Q, Chen X, Huang T, Liu M, Zhai Q. Angiogenic effects of apigenin on endothelial cells after hypoxia-reoxygenation via the caveolin-1 pathway. International Journal of Molecular Medicine. 2017; 40: 1639–1648.

[30] Sonveaux P, Martinive P, DeWever J, Batova Z, Daneau G, Pelat M, *et al*. Caveolin-1 expression is critical for vascular endothelial growth factor-induced ischemic hindlimb collateralization and nitric oxide-mediated angiogenesis. Circulation Research. 2004; 95: 154–161.

[31] Margheri F, Chillà A, Laurenzana A, Serratì S, Mazzanti B, Saccardi R, *et al*. Endothelial progenitor cell-dependent angiogenesis requires localization of the full-length form of uPAR in caveolae. Blood. 2011; 118: 3743–3755.

[32] Chidlow JH Jr, Sessa WC. Caveolae, caveolins, and cavins: complex control of cellular signalling and inflammation. Cardiovascular Research. 2010; 86: 219–225.

[33] Rudnicka E, Suchta K, Grymowicz M, Calik-Ksepka A, Smolarczyk K, Duszewska AM, *et al*. Chronic Low Grade Inflammation in Pathogenesis of PCOS. International Journal of Molecular Sciences. 2021; 22: 3789.

[34] Tarkun I, Arslan BC, Cantürk Z, Türemen E, Sahin T, Duman C. Endothelial dysfunction in young women with polycystic ovary syndrome: relationship with insulin resistance and low-grade chronic inflammation. The Journal of Clinical Endocrinology and Metabolism. 2004; 89: 5592–5596.

[35] Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and

long-term health risks related to polycystic ovary syndrome. Fertility and Sterility. 2004; 81: 19–25.

[36] Jeong JH, Ojha U, Lee YM. Pathological angiogenesis and inflammation in tissues. Archives of Pharmacal Research. 2021; 44: 1–15.

[37] Andleeb F, Asif M, Yaseen HS. Exploration of molecular mechanisms responsible for anti-inflammatory and anti-angiogenic attributes of methanolic extract of Viola betonicifolia. Inflammopharmacology. 2022; 30: 1459–1474.

[38] Li B, Vincent A, Cates J, Brantley-Sieders DM, Polk DB, Young PP. Low levels of tumor necrosis factor alpha increase tumor growth by inducing an endothelial phenotype of monocytes recruited to the tumor site. Cancer Research. 2009; 69: 338–348.

[39] Al Madhoun A, Kochumon S, Haddad D, Thomas R, Nizam R, Miranda L, *et al*. Adipose Tissue Caveolin-1 Upregulation in Obesity Involves TNF-α/NF-κB Mediated Signaling. Cells. 2023; 12: 1019.