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Original Article

Abnormal expression of galectin-1, -3 leading to unexplained infertility by decreasing endometrial receptivity: a retrospective analysis

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Abstract: Objective: To explore the relationship between galectin-1, -3 and unexplained infertility and the effect on endometrial receptivity. Methods: The clinical data of 100 female patients at childbearing age coming to Xingtai People's Hospital from February 2019 to February 2021 were collected retrospectively. Based on normal pregnancy or not, 50 infertility patients were placed into an infertility group, and 50 patients with normal pregnancy history were placed into a normal group. The mRNA and protein levels of galectin-1, -3, endometrial wave-like activity, endometrial thickness, uterine artery pulsatility index (PI), resistance index (RI), end diastolic velocity (EDV) and peak systolic velocity (PSV) ratio (S/D = PSV/EDV) were compared between the two groups of patients. Results: The mRNA and protein levels of galectin-1, -3 in the infertile group were lower than those in the normal group ($P<0.05$). In addition, the endometrial wave-like activity in the infertile group was more than that in the normal group ($P<0.05$). The endometrial thickness was less, while PI, RI and S/D were higher in the infertile group than those in the normal group ($P<0.05$). Conclusion: The low mRNA and protein expressions of galectin-1, -3 in unexplained infertility can affect endometrial receptivity, which may be closely related to unexplained infertility.

Keywords: Galectin-1, -3, unexplained infertility, endometrial receptivity

Introduction

Unexplained infertility refers to infertility that has not been found with a clear cause after detailed examination of infertility, and it accounts for about 10% of the total infertility in the population [1]. The poor receptivity of the endometrium during the implantation window for fertilized eggs may be one of the main causes of unexplained infertility [2]. A large amount of evidence has proven that the occurrence of infertility and spontaneous abortion is closely related to the low endometrial receptivity [3]. The introduction of assisted reproductive technology has provided greater possibilities for the treatment of infertility, but at present, its success rate is only 20%-35%, mainly because the formation of endometrial receptivity is defective or delayed [4]. Therefore, understanding endometrial receptivity is of great importance for the evaluation of infertility.

Endometrial receptivity mainly refers to the functional and morphological changes of the endometrium, which provides a comfortable and suitable intrauterine environment for embryo implantation [5]. Endometrial receptivity is the ability to accept embryos in every menstrual cycle, but it is regulated and restricted by many factors [6]. This period is clinically called the implantation window, mainly 6-10 days after ovulation. Clinically, it is used to evaluate endometrial receptivity, and the commonly used method is B-ultrasound [7]. Generally, when the endometrium of a patient during ovulation is over 8 cm, it means that the endometrial receptivity is good, indicating that the endometrium has three-line signs and is suitable for embryo implantation [8]. When the endometrial blood flow index PI detected by B-ultrasound is less than 2, it indicates that the endometrial receptivity is poor. In addition to B-ultrasound detection, the endocytosis of the patient's endometrium can be observed clini-

cally by electron microscopy, which can also effectively detect the receptivity of the endometrium [9]. The establishment mechanism of endometrial receptivity is very complex, and many factors participate in the regulation. These factors include estrogen and progesterone secreted by ovaries, embryogenic factors secreted by embryos and endometrium, local molecules of endometrium, etc. [10]. Hormones secreted by ovaries are macro-control factors for the formation of endometrial receptivity, and embryos and the endometrium periodically express receptivity related molecules, thus promoting the establishment of receptivity [11].

Lectins are a class of proteins that can selectively recognize and non-covalently bind to sugar structures β-Galactoside, and the oligosaccharide structure recognized by galactoside exists in most glycoproteins [12]. S-type coagulant, namely galectin, is widely distributed in many tissues and cells. It plays a biological role through the classical receptor ligand action mode in pathological and physiological aspects [13]. A lot of evidence has shown that glycosidic bonds play an important role in the formation of mammalian embryos. Galectin-1, -3 has received increasing attention in the field of reproduction [14].

Unexplained infertility is when the cause of infertility cannot be clearly identified by current examination methods, and the obstacle of fertilized egg implantation may be one of the main reasons, which may be related to endometrial receptivity during the window of fertilized egg implantation [15]. Endometrial receptivity is closely related to embryogenic factors and progesterone secreted by embryos. Galectin is widely distributed in cells and tissues and participates in immune regulation, apoptosis, cell adhesion and other processes. Some studies have found that galectin participates in the whole process of fertilization and embryo formation [16]. The purpose of this paper is to study the relationship between galectin-1, -3 and unexplained infertility, and the effect on decreasing endometrial receptivity in infertile patients. The innovation of this study is to prove that the mRNA and protein expressions of galactin-1, -3 are closely related to unexplained infertility, and the expression of galactin-1, -3 is related to endometrial receptivity, which could provide a basis for the evaluation of unexplained infertility.

Materials and methods

The general data

The clinical data of 100 female patients at childbearing age coming to Xingtai People's Hospital from February 2019 to February 2021 were collected retrospectively. Based on normal pregnancy or not, 50 infertility patients were placed into an infertility group, and 50 patients with normal pregnancy history were placed into a normal group. This study was approved by the Ethics Committee of Xingtai People's Hospital.

All the patients in the infertility group met the following criteria: (1) Women of childbearing age (aged 20-40 years); (2) Women who had normal sexual life after marriage without contraception; (3) Women who had been cohabiting for two years without pregnancy; (4) Women with regular menstrual cycle, normal biphasic temperature pattern, normal hormone levels, normal size of uterus and unobstructed fallopian tubes on both sides; (5) Women whose male partner had a normal semen examination result; (6) Women who were diagnosed with female unexplained infertility via exclusion of anatomy, infection and other factors.

All the patients in the normal group met the following criteria: (1) Women of childbearing age: aged 20-40 years; (2) Women who had a history of one or more normal pregnancies; (3) Women who had regular menstrual cycles; (4) Women without endocrine, immune or metabolic diseases, and didn't receive hormone treatment within 3 months.

Exclusion criteria of the two groups: (1) Those with organic diseases of uterus or ovary; (2) Those with blocked fallopian tubes; (3) Those with incomplete data records.

Endometrial sampling

Before endometrial sampling, informed consent was obtained from the patients. The B-ultrasound monitoring was carried out around the tenth day of the menstrual cycle to observe the endometrium and follicles in the body. One week after ovulation, the patient underwent B-ultrasound again to monitor the thickness of the endometrium. Before taking the endometrium, the patient's vagina was rinsed with 0.9% sodium chloride solution,

which does not dilate the cervix. After washing, a 3 mm small spatula was used to scratch the uterus to collect the endometrial tissue in a gentle and slow way to avoid damage. If the tissue collection caused any bleeding, it is necessary to rinse the endometrium gently with 0.9% sodium chloride solution, and then the tissue was put into 10% formaldehyde solution for fixation. The fixation time was less than 24 hours, and then the tissue was paraffin embedded.

Measuring mRNA and protein levels of galectin-1, -3

Experimental methods: The mRNA levels of galectin-1, -3 were measured by reverse transcription polymerase chain reaction (RT-PCR): (1) Extraction of galectin-1, -3 RNA; (2) Galectin-1, -3 RNA quality detection; (3) Synthesis of galectin-1, -3 cDNA; (4) A housekeeping gene in a gradient diluted standard and sample was tested (β-action) by real time quantitative PCR; (5) Preparation of DNA a template for plotting gradient dilution standard curve; (6) Real time quantitative PCR of galectin-1, -3 RNA.

The protein levels of galectin-1, -3 were measured by immunohistochemistry: (1) Slide washing; (2) Embedding organization; (3) Section; (4) Salvage organization; (5) Dewaxing; (6) Antigen repair; (7) Serologic blockade; (8) Add mouse anti human galectin-3 monoclonal antibody (Brand: LSBio; Article No.: LS-C62616); (9) Culture with mouse anti human vascular endothelial growth factor/vascular endothelial growth factor (VEGF/VPF) monoclonal antibody; (10) Culture with horseradish peroxidase labeled streptomycin ovalbumin working solution (Brand: Yaji Biology; Article No.: IH-2037R); (11) Culture with developer; (12) Re-dyeing; (13) Dehydration; (14) Cover.

Result evaluation: Negative control: the PBS solution was used as the negative control.

Positive results: all sections were read and recorded by pathologists in a double blind manner. Item A: Positive cell staining intensity was scored as follows, colorless: 0 point, light yellow: 1 point, yellow-brown: 2 points, brown: 3 points. Item B: The proportion of positive cells was scored as follows: negative = 0 points; positive cells ≤10% = 1 point; positive cells 11%-50% = 2 points; positive cells 51%-75% = 3 points; positive cells ≥75% = 4 points. Score of each case = multiplication of two scores (A × B). A score ≥3 points indicated positive immune reaction.

Ultrasonic examination

The color Doppler ultrasound diagnostic instrument (Mindray resona8) was used to measure the size of uterine body and the thickness of endometrium, and evaluate in detail the wave-like activity and types of the endometrium, including positive motion, negative motion, opposite motion, irregular motion and no motion.

Observation indexes

1. Clinical data; 2. The mRNA levels of galectin-1, -3; 3. The protein levels of galectin-1, -3; 4. The endometrial wave-like activity; 5. Parameters from transvaginal ultrasound; (1) Endometrial thickness; (2) Uterine artery pulsatility index (PI); (3) Resistance index (RI); (4) End diastolic velocity (EDV) and peak systolic velocity (PSV) ratio (S/D = PSV/EDV).

Statistical analysis

SPSS 26.0 was applied to process the data. The counting data were shown as percentage and compared using χ^2 test. The measurement data were shown by mean ± SD, and compared by t-test. The difference was significant if $P<0.05$.

Results

Baseline characteristics

There was no obvious difference in the age and body mass index between the two groups, indicating group comparability ($P>0.05$). In addition, the average infertile time in the infertility group was (6.64±2.90) years (**Table 1**).

The mRNA levels of galectin-1, -3 in the infertility and normal groups

The mRNA levels of galectin-1, -3 in the infertility group were lower than those in the normal group ($P<0.05$) (**Figure 1**).

The protein levels of galectin-1, -3 in the infertility and normal groups

The protein levels of galectin-1, -3 in the infertility group were lower than those in the normal group ($P<0.05$) (**Figure 2**).

Table 1. Baseline characteristics

Index	Infertility group (n = 50)	Normal group (n = 50)	t	P
Age (years old)	31.52±3.44	31.88±3.35	-0.53	0.597
BMI (kg/m^2)	20.94±1.06	20.96±1.10	-0.069	0.945
Infertile time (years)	6.64±2.90	-	-	-

Note: Body mass index (BMI).

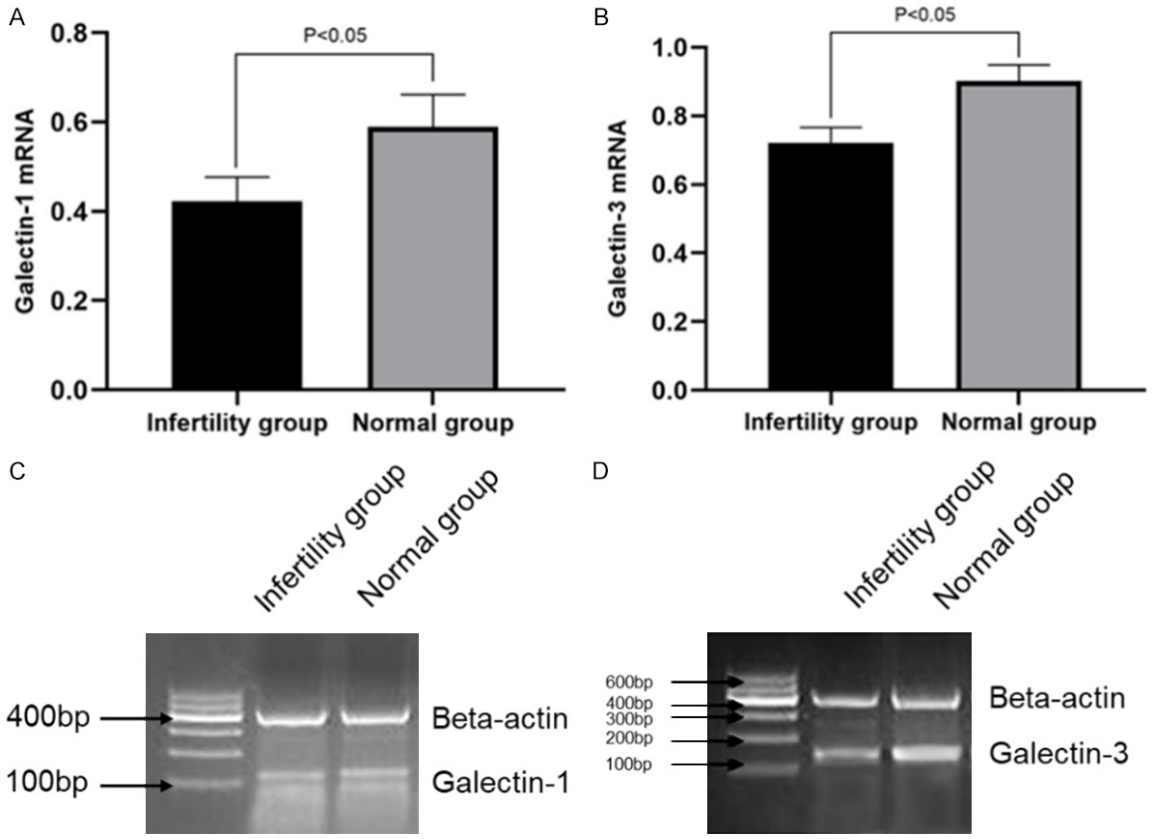

Figure 1. The mRNA levels of galectin-1 and galectin-3 in the infertility and normal groups. A, C: Galectin-1 mRNA; B, D: Galectin-3 mRNA.

Endometrial wave-like activity

The endometrial wave-like activity in the two groups was observed and compared. The activity in the infertility group was more than that in the normal group (P<0.05). See **Figure 3**.

Parameters detected by transvaginal ultrasound

As shown in **Figures 4** and **5**, the changes of parameters detected by transvaginal ultrasound in the two groups were observed and compared. The endometrial thickness in the infertility group was less than that in the normal group, and the PI, RI and S/D were higher in the infertility group than those in the normal group (P<0.05).

Discussion

Spontaneous abortion is a common and frequently occurring disease among women of gestational age. The rate of spontaneous abortion accounts for 10-15% of all pregnancies, and over 80% are early abortions [17]. Current research has shown that genetic factors, endocrine factors, infection factors are inducing factors of spontaneous abortion [18]. In addition, some causes are unknown. A study showed that the expression of galectin-1 protein in the embryo group of early abortion was lower

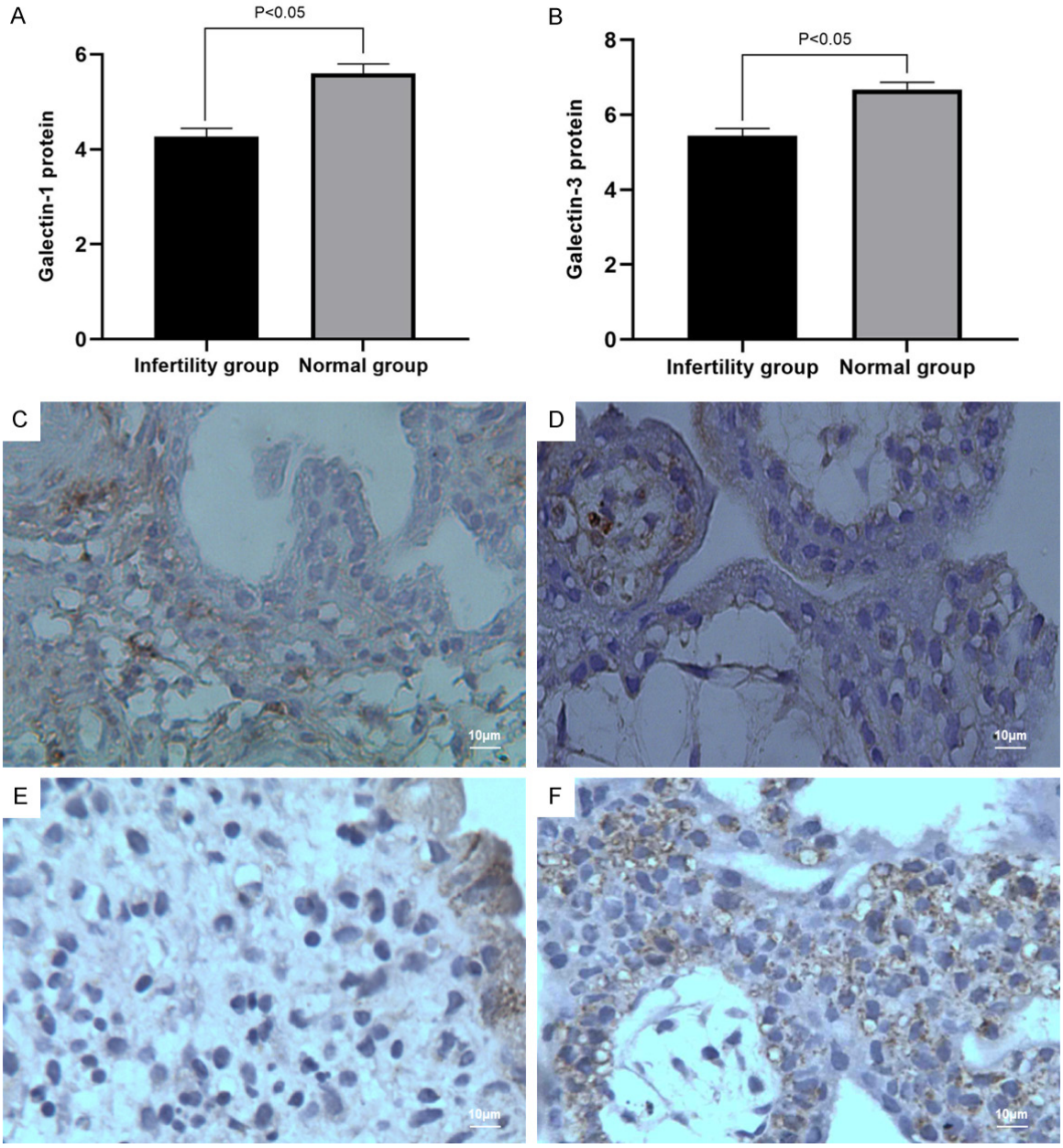

Figure 2. The protein levels of galectin-1 and galectin-3 in the infertility and normal groups. A: Galectin-1 protein; B: Galectin-3 protein; C: Galectin-1 protein in the infertility group; D: Galectin-1 protein in the normal group; E: Galectin-3 protein in the infertility group; F: Galectin-3 protein in the normal group; Scale bar, 10 μm.

than that of normal pregnancy, and the mice with galectin-1 gene deletion had a higher abortion rate than normal mice [19, 20], showing that galectin-1, -3 were closely related to unexplained early abortion.

Our results showed that the mRNA and protein levels of galactin-1, -3 in the infertility group were low. The high expression of galectin-1, -3 in gestational trophoblast indicates that galectin participates in the invasion and infiltration of blastocyst trophoblast into endometrium [21]. β-Galactoside is the main component of extracellular matrix. The combination of Galactosyl-3 and β-Galactoside can smoothly invade the endometrial stroma and blood vessels, so that the fertilized egg can be successfully implanted [22]. This explains why low expression of galactin-1, -3 may lead to infertility.

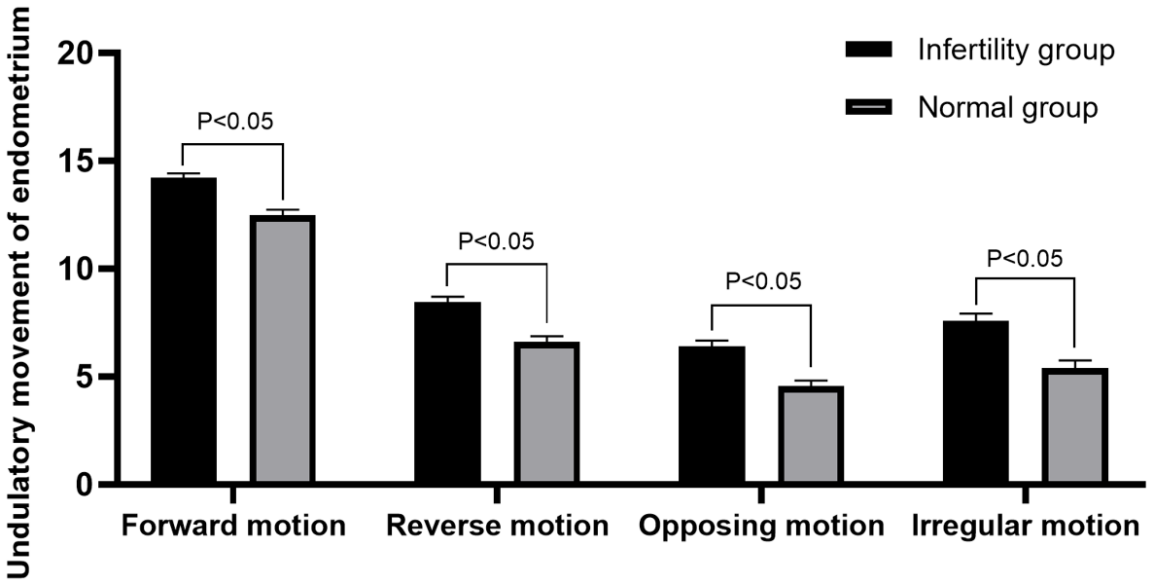

Figure 3. Endometrial wave-like activity.

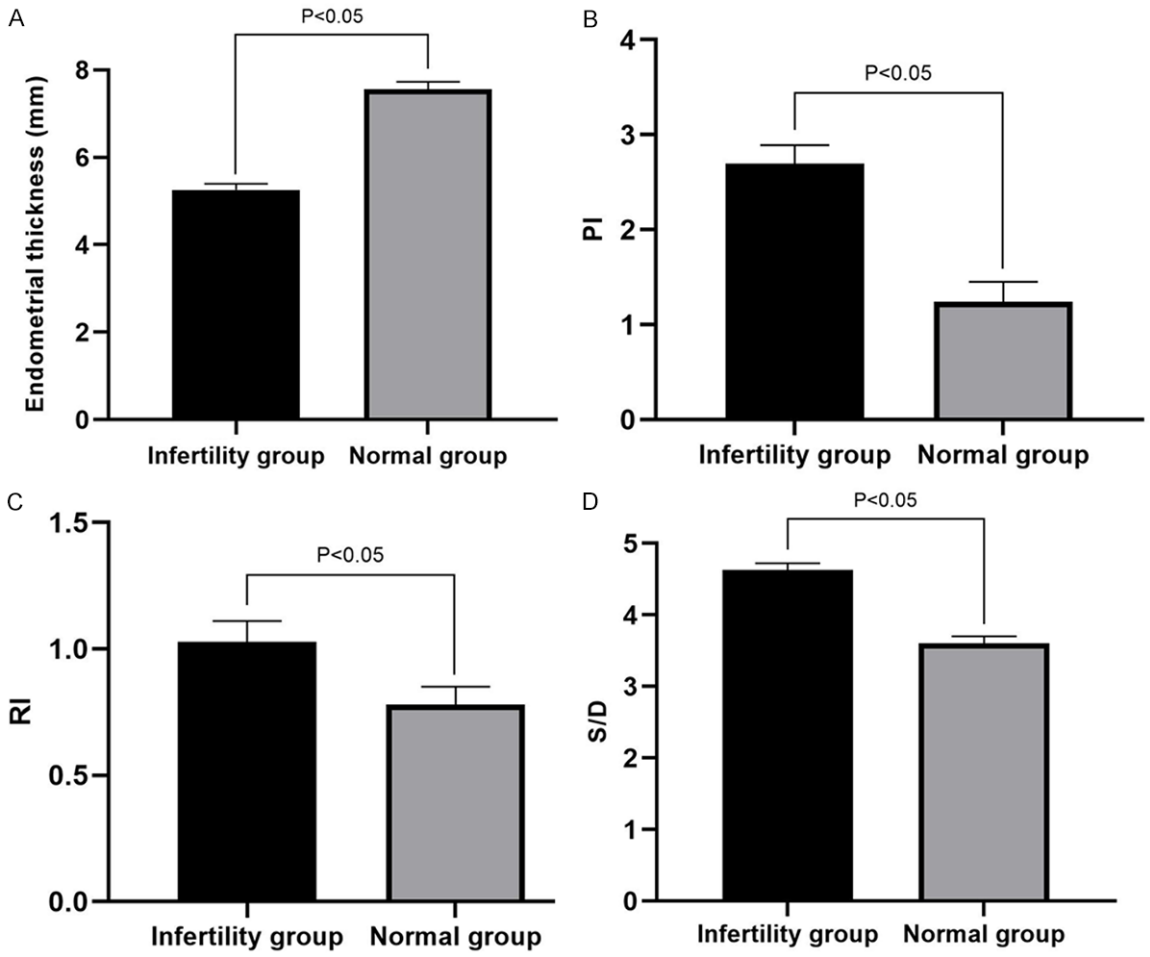

Figure 4. Parameters detected by transvaginal ultrasound. A: Endometrial thickness; B: Uterine artery PI; C: RI; D: S/D. Note: PI, pulsatility index; RI, resistance index; EDV, end diastolic velocity; PSV, peak systolic velocity; S/D = PSV/EDV.

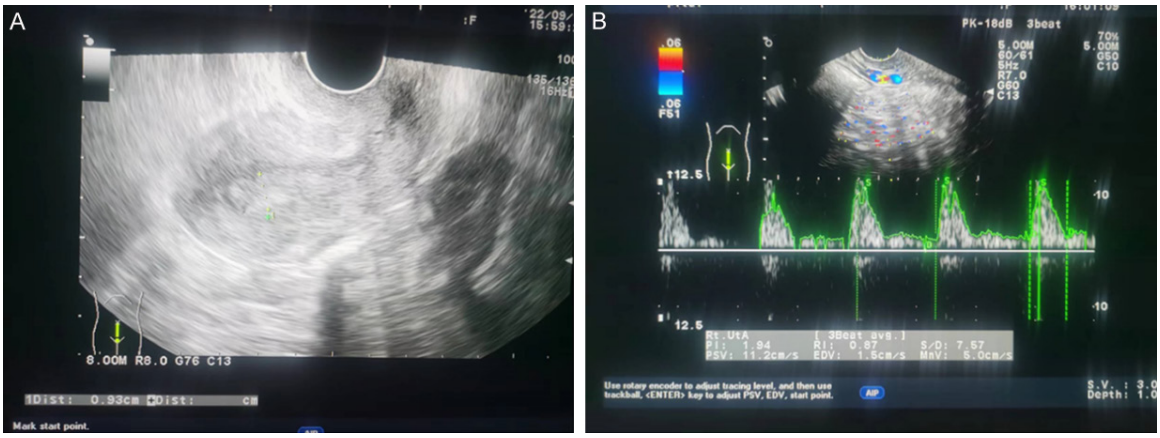

Figure 5. Transvaginal ultrasound of infertile group. A: Endometrial thickness; B: Uterine artery PI, RI and S/D. Note: PI, pulsatility index; RI, resistance index; EDV, end diastolic velocity; PSV, peak systolic velocity; S/D = PSV/EDV.

In addition, the endometrial wave-like activity in the infertility group was higher than that in the normal group. The endometrial thickness, PI, RI and S/D showed a poor presence in the infertility group. These results suggest that galectin-1, -3 may cause infertility by reducing endometrial receptivity. In patients with infertility, hormones lack periodic changes, while uterine blood flow and uterine function change due to the influence of ovarian hormone level. They are synergistic. RI value may be related to blood viscosity, friction between blood cells, increased friction between blood vessel walls, and vascular status [23]. Therefore, uterine hemodynamics were in disorder, and RI of uterine spiral artery and S/D were significantly increased, presenting low amplitude discontinuous waves, affecting normal blood supply, which is not conducive to implantation [24].

In this study, we explored the relationship between galectin-1, -3 and unexplained infertility and the effect on endometrial receptivity. The low mRNA and protein levels of galectin-1, -3 in unexplained infertility can affect endometrial receptivity, which may be closely related to unexplained infertility and provide basis for the diagnosis and treatment of unexplained infertility. However, there are some limitations in this study. Firstly, the sample size in this study is small. Secondly, the current physical and mental status of the research subject may affect the authenticity and accuracy of the past data reports, since our research is a retrospective analysis. It is necessary to design a prospective clinical trial and expand the scope of the study population, including the number of subjects and their areas, to evaluate the related indexes and explore the mechanism.

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Disclosure of conflict of interest

None.

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