EXPRESSION PROFILES OF GALECTIN-1 AND GALECTIN-3 IN THE ENDOMETRIUM OF UNEXPLAINED INFERTILITY PATIENTS

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ABSTRACT

Objective: *To explore the genetic and protein expression of galectin-1 and galectin-3 in the endometrium of patients with unexplained infertility, aiming to provide theoretical evidence for clinical diagnosis of unexplained infertility.*

Methods: *In Xingtai People's Hospital, a total of 100 patients with the clinical diagnosis of unexplained infertility were enrolled into the observation group between February 2019 and September 2021. During the same period, 40 subjects who received the placement of intrauterine rings within 1 year after normal delivery and 60 patients with normal pregnancy history who underwent the surgical treatment for simple cyst of ovary or paroophoritic cyst were recruited into the control group. The mRNA and protein expression of galectin-1 and galectin-3 in specimens collected from subjects above were detected via the reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry staining.*

Results: *Significant differences were identified in the expression of mRNA and protein of galectin-1 and galectin-3 between the normal endometrium and endometrium of patients with unexplained infertility; in normal endometrium, mRNA expression of galectin-1 and galectin-3 was significantly higher than that in the endometrium at the advanced stage of hyperplasia ($P<0.05$); in endometrium of patients with unexplained infertility, mRNA expression in the middle stage of secretion had no significant difference compared to that in the advanced stage ($P>0.05$). However, in the normal endometria or those of patients with unexplained infertility, mRNA expression of galectin-3 was much higher than that of galectin-1 ($P<0.05$).*

Conclusion: *The mRNA of galectin-1 and galectin-3 may alter the embryo implantation by affecting the tolerance of endometrium.*

Keywords: *Endometrium, infertility, Galectin-1, Galectin-3.*

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Introduction

It has been estimated that about 10-15% of couples are suffering from infertility in the world[(1)]. Most of the infertility cases are attributed to the obstruction of fallopian tubes, ovulatory dysfunction, endometriosis, an insufficient reserve of oocytes, and poor quality of seminal fluid[(2-3)]. However, for some patients with unexplained infertility (nearly 10% of infertility patients), the results of modern medical examination reveal that tubal patency, parameters of semen, ovulation, ovarian reserve, and endometrial cavity are normal, with no evident cause for infertility[(4)]. The process that blastocyst at the advanced stage is implanted into the endometrium is called the implantation of a fertilized egg. The fertilized egg develops into the advanced stage, gaining the implantation ability. Meanwhile, endometrium also attains the accepting status, thereby gaining the ability to accept the blastocyst implantation, i.e. the tolerance of endometrium[(5-6)]. Thus, poor tolerance of endometrium and disorder in implantation of the fertilized egg would result in unexplained infertility. The Galectin family, as a group of proteins that are able to recognize the structure of galactose specifically, is involved in various pathophysiological processes, including cell apoptosis, inflammation, immunoregulation,

metastasis, and infiltration of tumors[7-9]. Galectin-1 and galectin-3, mainly expressed in the cells of the middle layer, distal end, and syncytiotrophoblasts, can alter the implantation of the blastocyst and adjust the tolerance of endometrium through mediating the cell-cell, cell-extracellular matrix adhesion, promoting cell infiltration and formation of syncytial cells and affecting the artery formation, which, thus, could be served as one of the major indexes for evaluation of the tolerance of endometrium[10-12].

As such, in this study, we explored the expression profile of galectin-1 and galectin-3 in the endometrium of patients with unexplained infertility and that of normal subjects, and the results are expected to provide theoretical evidence for clinical diagnosis of unexplained infertility.

Materials and methods

Subjects

All specimens were collected from the patients who visited the clinic or were admitted to Xingtai People's Hospital between February 2019 and September 2021. Endometrium was collected by using the uteroscope and confirmed pathologically as the normal endometrium. Among those subjects, there were 100 patients who were diagnosed with unexplained infertility, including 50 patients in the advanced stage of proliferation (from the 11th day to the 15th day during a menstrual cycle) and 50 in the middle stage of secretion (from the 20th day to 24th day during a menstrual cycle). During the same period, 40 subjects who received the placement of intrauterine rings within 1 year after normal delivery and 60 patients with normal pregnancy history who underwent the surgical treatment for simple cyst of the ovary or paroophoritic cyst were recruited into the control group.

Inclusion criteria:

• Women in the child-bearing period (19-41 years old), with regular menstruation but no dysmenorrhea, placement of the intrauterine device or adverse history of pregnancy, and at menolipsis of 50 ± 5 days at the time of enrollment.

Comparison of the age of patients between the two groups showed no significant difference, suggesting that their ages were comparable.

Major facilities and reagents

Equipment

Refrigerator, microtome, microwave oven, air blower, IHC pen, wet cassette, oven, oscillator, dye vat, microscope, timer, and fuming cupboard.

Reagents

Rat anti-human monoclonal anti-galectin-3 antibody, rat anti-human monoclonal anti-VEGF/VPF monoclonal antibody, SP kit, PBS solution, horseradish peroxidase (HRP)-labeled streptomycin ovalbumin working solution.

Methods

Reverse transcription-polymerase chain reaction and immunohistochemistry staining were carried out to determine the mRNA and protein expression of galectin-1 and galectin-3 in all collected specimens. All specimens were divided into two parts: one part, within 20 min after collection, was fixed in10% paraformaldehyde overnight, followed by the dehydration in ethanol, clearing in xylene, embedding in paraffin, and slicing, and the other part was placed into the RNase-free EP tube after being rinsed in DEPC-normal saline to remove the blood and then placed in -80°C for later use (For cases that failed to be preserved in -80°C promptly, they were stored in RNA preserving fluid to prevent the degradation of RNA).

Target genes	Primers	Amplification
LGASL1	Upstream: 5'--AGCGGGAGGCTGTCTTTC-3' Downstream: 5'--TCCAGGTGGAGGCGGTTG-3'	129bp
LGASL3	Upstream: 5'--GTGCCTTATAACCTGCCTTTG-3' Downstream: 5'--GACTCTCCTGTTGTTCTCATT-3'	164bp
β-actin	Upstream: 5'--CGGGAAATCGTGCTGGAC-3' Downstream: 5'--TGGAAGGTGGACAGCGAG-3'	416bp

Table 1: Primer sequences.

Evaluation of results

The primary antibody was replaced by PBS as the negative control. Positive results: Cells and tissues are structurally clear, with brown or deep brown particles in the cytoplasm and staining intensity higher than the background.

All sections were reviewed and recorded by two qualified pathologists independently in a double-blind manner. For each section, 200 cells were counted from 10 non-overlapped visions under the high-power lens.

Results were evaluated according to the evaluation criteria of immunohistochemistry results of Xu ZL et al.[2], to measure the percentage and signal strength of positive cells in all cells:

• Item A: signal strength of positive cells: 0 for no signal, 1 point for positive cells with pale yellow

stain, 2 points for pale brown stain and 3 points for brown stain;

• Item B: percentage of positive cells: 0 for negative, 1 point for percentage of positive cells ≤10 %, 2 points for percentage between 11% and 50%, 3 points for percentage between 51% and 75% and 4 points for percentage ≥75 %.

Staining score of each section is the product of Item A and Item B, and section with score ≥3 points was taken as immunoreactive cells.

Statistical methods

SPSS 13.0 software was utilized to perform the data analysis. All measurement data, including the intensity of bands and staining score, were expressed in form of mean ± standard deviation. All data were subjected to the normality test, and those conforming to the normal distribution were eligible for following analysis. To compare the mean of independent samples, the homogeneity test of variance was performed using t-test. To compare the means among multiple samples, the SNK analysis of variance was carried out.

For means of samples not conforming to the normal distribution, a non-parameter test was carried out. Correlation between the expression profiles of galectin-1 and galectin-3 was testified via the linear correlation analysis. Any difference at $P<0.05$ was statistically significant.

Results

Differences were noted in the mRNA and protein expression of galectin-1 and galectin-3 between the endometria of patients with unexplained infertility and normal endometria (Figures 1 and 2).

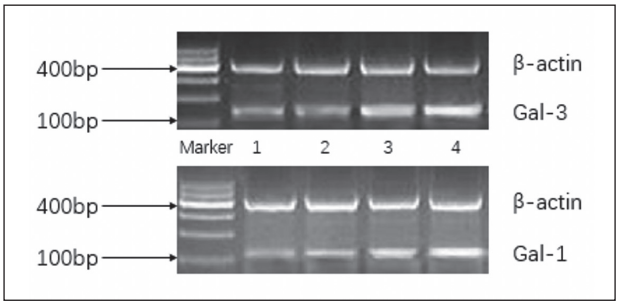

Figure 1: mRNA expression of galectin-1 and galectin-3 in different endometria.

Note: 1. Endometrium of patients with unexplained infertility at the advanced stage of proliferation; 2. Endometrium of patients with unexplained infertility at the middle stage of proliferation; 3. Endometrium of healthy subjects at the advanced stage of proliferation; 4. Endometrium of healthy subjects at the middle stage of proliferation.

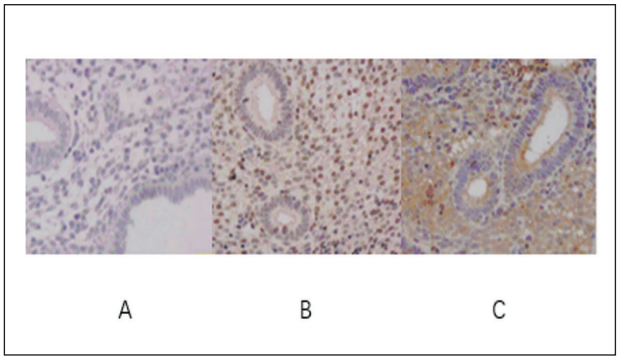

Figure 2: Protein expression of galectin-3 in the endometrium. A: Negative control; B: Healthy endometrium; C: Endometrium of patients with unexplained infertility.

In healthy endometrium, mRNA expression of galectin-1 and galectin-3 elevated significantly in the endometrium at the middle stage of secretion as compared to those at the advanced stage of proliferation.

However, in the endometrium of patients with unexplained infertility, no significant difference was found in the mRNA expression of galectin-1 and galectin-3 in comparison with the endometrium at the advanced stage ($P>0.05$; Figure 3).

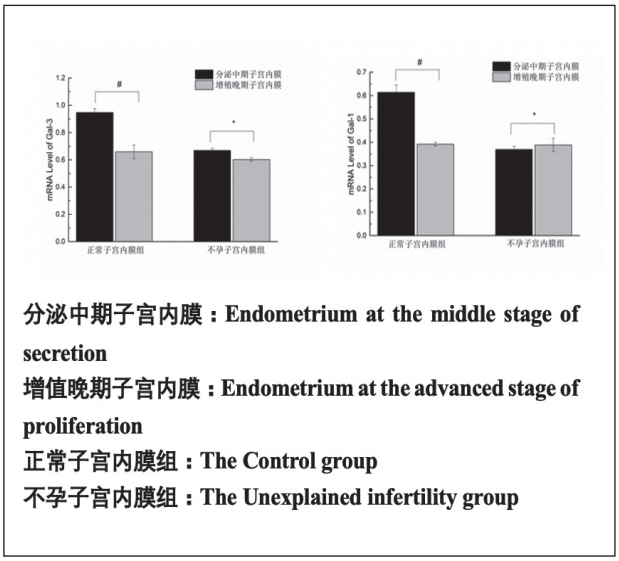

Figure 3: mRNA expression of galectin-1 and galectin-3 in the endometrium at the middle stage of secretion or the advanced stage of proliferation.

*#P<0.001; *P>0.05.*

In the endometria collected from patients with unexplained infertility or healthy subjects, mRNA expression of galectin-3 was in positive correlation with that of galectin-1, and the mRNA expression of galectin-3 was much higher than that of galectin-1 (Figure 4).

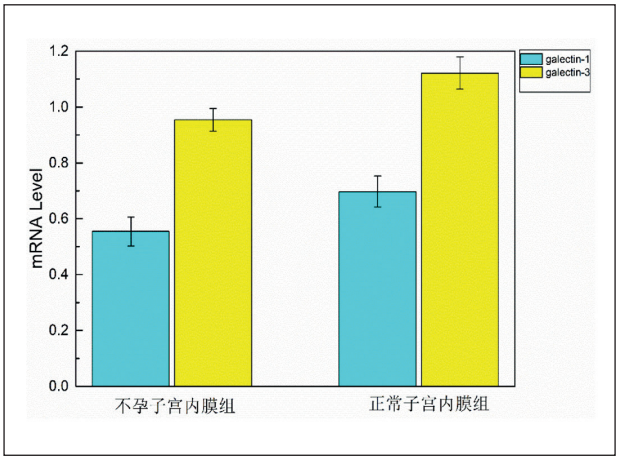

Figure 4: Correlation between the mRNA expression of galectin-3 and galectin-1 in different Endometria.

Discussion

Galectins, as a group of carbohydrate-binding proteins, have been reported to possess the pro-inflammatory or anti-inflammatory effects on reproductive tract or the pathological conditions related to infertility[13]. Galectin, as reported, is expressed extensively in reproductive tissues: Galectin-1, 3, 9, and 15 have been found to be expressed in the human endometrium and decidua[14]. Thus, galectins may act pivotally in the immunoregulation system of the endometrium by interfering in cell adhesion, migration, and chemotaxis[15]. Galectin-1, as a kind of homodimer, is composed of subunit (MWM = 14.5 kDa). It can facilitate cell proliferation and motion by binding to the extracellular matrix[16]. Galectin-1 is also reported to be the pro- or anti-inflammatory cytokines to be involved in the inflammation[17]. Generally, galectin-1 is expressed in the endometrium and decidua, which plays a key role in implantation and trophoblast invasion[18].

Decidua and implantation are affected by various factors of endocrine, paracrine and autocrine, like hormones, cytokines, and growth factors[19]. Expression profiles of galectin-1 are similar in the placenta and extra-embryonic membranes, which may correlate with the invasion mechanism of human trophoblasts[20]. In addition, galectin-1 is regulated by the ovarian steroid[21] to affect the implantation of blastocyst[22] and trophoblast invasion[23], thereby mediating the maternal-embryonic immunity/endocrine crosstalk and placentation[24, 25]. Moreover, galectin-1 is highly expressed in the human placenta in the advanced stage of pregnancy and extra-embryonic membranes and further up-regulated in the placenta of preeclampsia patients or extra-embryonic membranes of patients with chorioamnionitis, while down-regulated in the endometrium of patients after an abortion[26].

Galectin-3, also known as β-galactoside-binding lectin, is a kind of 31 kDa protein possessing the collagen-α-like domain, N-terminal domain, and C-terminal carbohydrate recognition domain, which permit galectin-3 to possess the specific biological functions, including cell adhesion and migration and extracellular cell-cell interaction[27], immune responses[28] and signal transduction[29]. It has been established that galectin-3 is expressed in a variety of cells, including the endometrial cells and trophoblasts[30,31]. Previous literature has reported that galectin-3 is specifically expressed in endometrial cells in secretion[32, 33], placenta at the early stage of pregnancy and decidua surrounding the placentation. Recent evidence suggests that galectin-3 is correlated with the endometrium tolerance during the implantation[34-36]. Additionally, galectin-3 is thought to be a part of nuclei and cytoplasm that is able to pass through the nuclei and cytoplasm[37, 38]. Estrogen and progesterone, as a member of superfamily of nuclear horomes, are also found to be pivotal to the formation of accepting endometrium[39]. Results of this study demonstrated that in endometrium of patients with unexplained infertility, mRNA and protein expression of galectin-1 and galectin-3 in the middle stage of secretion had no significant difference when comparing to that in the advanced stage ($P>0.05$). Therefore, tolerance of endometrium is poor and not affected by the menstrual cycle.

In conclusion, the mRNA of galectin-1 and galectin-3 may alter the embryo implantation via affecting the tolerance of endometrium. However, there remains no exact evidence suggesting that any down-regulation of galectin-1 and galectin-3 could result in infertility. Thus, how galectin-1 and galectin-3 alter the tolerance of endometrium requires further studies.

References

1) Stewart JD, Pasternak MC, Pereira N, Rosenwaks Z. Contemporary Management of Unexplained Infertility. Clin Obstet Gynecol. 2019 Jun; 62(2): 282-292. doi: 10.1097/GRF.0000000000000450. PMID: 30994483.
2) Ray A, Shah A, Gudi A, Homburg R. Unexplained infertility: an update and review of practice. Reprod Biomed Online. 2012 Jun; 24(6): 591-602. doi: 10.1016/j.rbmo.2012.02.021. Epub 2012 Mar 7. PMID: 22503948.
3) Carson SA, Kallen AN. Diagnosis and Management of Infertility: A Review. JAMA. 2021 Jul 6; 326(1): 65-76. doi: 10.1001/jama.2021.4788. PMID: 34228062.
4) Carson SA, Kallen AN. Diagnosis and Management of Infertility: A Review. JAMA. 2021 Jul 6; 326(1): 65-76. doi: 10.1001/jama.2021.4788. PMID: 34228062.
5) Deroux A, Dumestre-Perard C, Dunand-Faure C, Bouillet L, Hoffmann P. Female Infertility and Serum Auto-antibodies: a Systematic Review. Clin Rev Allergy Immunol. 2017 Aug; 53(1): 78-86. doi: 10.1007/s12016-016-8586-z. PMID: 27628237.
6) Satwik R, Kochhar M. Unexplained infertility categorization based on female laparoscopy and total motile sperm count, and its impact on cumulative live-births after one in-vitro fertilization cycle. A retrospective cohort study involving 721 cycles. Reprod Med Biol. 2021 Feb 1; 20(2): 190-198. doi: 10.1002/rmb2.12368. PMID: 33850452; PMCID: PMC8022093.
7) Brophy ML, Murphy JE, Bell RD. Assessment of galactose-1-phosphate uridyltransferase activity in cells and tissues. J Biol Methods. 2021 Jun 30; 8(2): e149. doi: 10.14440/jbm.2021.355. PMID: 34258307; PMCID: PMC8270791.
8) Manero-Rupérez N, Martínez-Bosch N, Barranco LE, Visa L, Navarro P. The Galectin Family as Molecular Targets: Hopes for Defeating Pancreatic Cancer. Cells. 2020 Mar 11; 9(3): 689. doi: 10.3390/cells9030689. PMID: 32168866; PMCID: PMC7140611.
9) Pergialiotis V, Papoutsi E, Androutsou A, Tzortzis AS, Frountzas M, Papapanagiotou A, Kontzoglou K. Galectins-1, -3, -7, -8 and -9 as prognostic markers for survival in epithelial ovarian cancer: A systematic review and meta-analysis. Int J Gynaecol Obstet. 2021 Mar; 152(3): 299-307. doi: 10.1002/ijgo.13471. Epub 2020 Dec 25. PMID: 33156523.
10) Hisrich BV, Young RB, Sansone AM, Bowens Z, Green LJ, Lessey BA, Blenda AV. Role of Human Galectins in Inflammation and Cancers Associated with Endometriosis. Biomolecules. 2020 Feb 4; 10(2): 230. doi: 10.3390/biom10020230. PMID: 32033052; PMCID: PMC7072718.
11) Tang M, Zhu Y. [The involvement of galectin-1 in implantation and pregnancy maintenance at the maternal-fetal interface]. Zhejiang Da Xue Xue Bao Yi Xue Ban. 2017 May 25; 46(3): 321-327. Chinese. PMID: 29039177.
12) El-Azzamy H, Balogh A, Romero R, Xu Y, LaJeunesse C, Plazyo O, Xu Z, Price TG, Dong Z, Tarca AL, Papp Z, Hassan SS, Chaiworapongsa T, Kim CJ, Gomez-Lopez N, Then NG. Characteristic Changes in Decidual Gene Expression Signature in Spontaneous Term Parturition. J Pathol Transl Med. 2017 May; 51(3): 264-283. doi: 10.4132/jptm.2016.12.20. Epub 2017 Feb 22. PMID: 28226203; PMCID: PMC5445200.
13) Shimizu Y, Kabir-Salmani M, Azadbakht M, Sugihara K, Sakai K, et al. (2008) Expression andlocalization of galectin-9 in the human uterodome. Endocr J 55: 879-887.
14) Von Wolff M, Wang X, Gabius HJ, Strowitzki T (2005) Galectin fingerprinting in human endometrium and decidua during the menstrual cycle and in early gestation. Mol Hum Reprod 11: 189-194.
15) Kabir-Salmani M, Fukuda MN, Kanai-Azuma M, Ahmed N, Shiokawa S, et al. (2008) The membrane-spanning domain of CD98 heavy chain promotes alpha(v)beta3 integrin signals in human extravillous trophoblasts. Mol Endocrinol 22: 707-715.
16) Sun XF, Dai SY. The Significance of Galectin-1 and Galectin-9 Expression in Endometrial Carcinoma. Gynecol Obstet Invest. 2020; 85(1): 34-40. doi: 10.1159/000502787. Epub 2019 Sep 5. PMID: 31487707.
17) Yi L, Zhang S, Feng Y, Wu W, Chang C, Chen D, Chen S, Zhao J, Zhen G. Increased epithelial galectin-13 expression associates with eosinophilic airway inflammation in asthma. Clin Exp Allergy. 2021 Dec; 51(12): 1566-1576. doi: 10.1111/cea.13961. Epub 2021 Jun 19. PMID: 34075657.
18) Vergetaki A, Jeschke U, Vrekoussis T, Taliouri E, Sabatini L, Papakonstanti EA, Makrigiannakis A. Galectin-1 overexpression in endometriosis and its regulation by neuropeptides (CRH, UCN) indicating its important role in reproduction and inflammation. PLoS One. 2014 Dec 4; 9(12): e114229. doi: 10.1371/journal.pone.0114229. PMID: 25473847; PMCID: PMC4256414.
19) Mylonas I, Mayr D, Walzel H, Shabani N, Dian D, Kuhn C, Kunze S, Jeschke U, Friese K. Mucin 1, Thomsen-Friedenreich expression and galectin-1 binding in endometrioid adenocarcinoma: an immunohistochemical analysis. Anticancer Res. 2007 Jul-Aug; 27(4A): 1975-80. PMID: 17649808.
20) Maquoi E, van den Brûle FA, Castronovo V, Foidart JM. Changes in the distribution pattern of galectin-1 and galectin-3 in human placenta correlates with the differentiation pathways of trophoblasts. Placenta. 1997 Jul-Aug; 18(5-6): 433-9. doi: 10.1016/s0143-4004(97)80044-6. PMID: 9250706.
21) Carlos CP, Silva AA, Gil CD, Oliani SM. Pharmacological treatment with galectin-1 protects against renal ischaemia-reperfusion injury. Sci Rep. 2018 Jun 22;8(1):9568. doi: 10.1038/s41598-018-27907-y. PMID: 29934646; PMCID: PMC6015078.
[22] Colnot C, Fowlis D, Ripoche MA, Bouchaert I, Poirier F. Embryonic implantation in galectin 1/galectin 3 double mutant mice. Dev Dyn. 1998 Apr; 211(4): 306-13. doi: 10.1002/(SICI)1097-0177(199804)211:4<306:AID-AJA2>3.0.CO;2-L. PMID: 9566950.
23) Xu B, Shanmugalingam R, Chau K, Makris A, Hennessy A. Galectin-1-Related Modulation of Trophoblast Endothelial Interactions by Integrins α1 and β1. Reprod Sci. 2020 May; 27(5): 1097-1109. doi: 10.1007/s43032-019-00046-z. Epub 2020 Apr 6. PMID: 32253734.
24) Tang M, Zhu Y. [The involvement of galectin-1 in implantation and pregnancy maintenance at the maternal-fetal interface]. Zhejiang Da Xue Xue Bao

Yi Xue Ban. 2017 May 25; 46(3): 321-327. Chinese. PMID: 29039177.

25) Tirado-González I, Freitag N, Barrientos G, Shaikly V, Nagaeva O, Strand M, Kjellberg L, Klapp BF, Mincheva-Nilsson L, Cohen M, Blois SM. Galectin-1 influences trophoblast immune evasion and emerges as a predictive factor for the outcome of pregnancy. Mol Hum Reprod. 2013 Jan; 19(1): 43-53.

26) Wu M, Liu P, Cheng L. Galectin-1 reduction and changes in T regulatory cells may play crucial roles in patients with unexplained recurrent spontaneous abortion. Int J Clin Exp Pathol. 2015 Feb 1; 8(2): 1973-8. PMID: 25973091; PMCID: PMC4396310.

27) Dong R, Zhang M, Hu Q, Zheng S, Soh A, Zheng Y, Yuan H. Galectin-3 as a novel biomarker for disease diagnosis and a target for therapy (Review). Int J Mol Med. 2018 Feb; 41(2): 599-614. doi: 10.3892/ijmm.2017.3311. Epub 2017 Dec 5. PMID: 29207027; PMCID: PMC5752178.

28) Barondes SH, Cooper DN, Gitt MA et al (1994) Galectins.Structure and function of a large family of animal lectins. J BiolChem 269: 20807-20810.

29) Panjwani N (2014) Role of galectins in re-epithelialization of wounds. Ann Transl Med 2: 89.

30) Rabinovich GA, Liu FT, Hirashima M (2007) An emerging role for galectins in tuning the immune response: lessons from experimental models of inflammatory disease, autoimmunity and cancer. Scand J Immunol 66: 143-158.

31) Merlin J, Stechly L, de Beaucé S et al (2011) Galectin-3 regulates MUC1 and EGFR cellular distribution and EGFR downstream pathways in pancreatic cancer cells. Oncogene 30: 2514-2525. doi:10.1038/onc.2010.631.

32) Jovanović Krivokuća M, Vilotić A, Nacka-Aleksić M, Pirković A, Ćujić D, Legner J, Dekanski D, Bojić-Trbojević Ž. Galectins in Early Pregnancy and Pregnancy-Associated Pathologies. Int J Mol Sci. 2021 Dec 22; 23(1): 69. doi: 10.3390/ijms23010069. PMID: 35008499; PMCID: PMC8744741.

33) Al-Maghrabi J, Abdelrahman AS, Ghabrah T, Butt NS, Al-Maghrabi B, Khabaz MN. Immunohistochemical expression of galectin-3 is significantly associated with grade, stage and differentiation of endometrial carcinomas. Pathol Res Pract. 2017 Apr; 213(4): 348-352. doi: 10.1016/j.prp.2017.01.012. Epub 2017 Jan 19. PMID: 28215640.

34) Boutas I, Kontogeorgi A, Dimitrakakis C, Kalantaridou SN. The expression of Galectin-3 in endometrial cancer: a systematic review of the literature. Mol Biol Rep. 2021 Jul;48(7):5699-5705. doi: 10.1007/s11033-021-06536-1. Epub 2021 Jul 9. PMID: 34241773.

[35] Lei CX, Zhang W, Zhou JP, Liu YK. Interactions between galectin-3 and integrinbeta3 in regulating endometrial cell proliferation and adhesion. Hum Reprod. 2009 Nov; 24(11): 2879-89. doi: 10.1093/humrep/dep250. Epub 2009 Jul 24. PMID: 19633306.

36) Yang H, Yin J, Ficarrotta K, Hsu SH, Zhang W, Cheng C. Aberrant expression and hormonal regulation of Galectin-3 in endometriosis women with infertility. J Endocrinol Invest. 2016 Jul; 39(7): 785-91. doi: 10.1007/s40618-016-0435-7. Epub 2016 Feb 17. PMID: 26886939; PMCID: PMC4906070.

37) Hisrich BV, Young RB, Sansone AM, Bowens Z, Green LJ, Lessey BA, Blenda AV. Role of Human Galectins in Inflammation and Cancers Associated with Endometriosis. Biomolecules. 2020 Feb 4; 10(2): 230. doi: 10.3390/biom10020230. PMID: 32033052; PMCID: PMC7072718.

38) Nasrolahi S, Mohammadi Y, Abbasalipourkabir R, Kamkari S.; Comparison of serum VEGF and PLGF levels in Preeclamptic and healthy pregnant women in Hamadan, Iran. Perinatology Journal. 2018; 19(2): 76-81.

39) Yavangi Mahnaz, Mohammadi Ilnaz, Khansari Shahedeh, Moradkhani Shirin, Artimani Tayebe, Heidari Soureshjani Saeid. The Effects of Foeniculum vulgare Seed Extract on Fertility Results of Assisted Reproductive Technology in Women with Poor Ovarian Response. INTERNATIONAL JOURNAL OF WOMEN'S HEALTH AND REPRODUCTION SCIENCES. 2020: 8(2): 203-208. Available from: https://www.sid.ir/en/journal/ViewPaper.aspx?id=775949.

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