Novel Bio-Markers In Diagnosis Of Endometriosis

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TRICHY & PATTUKOTTAI

Greetings From Trichy….

AaKRITI

Presents

2025

ENDOMETRIOSIS – NEW HORIZONS

Endometriosis

Remains a riddle wrapped in a mystery in a enigma

Emery Wilson

Highlights

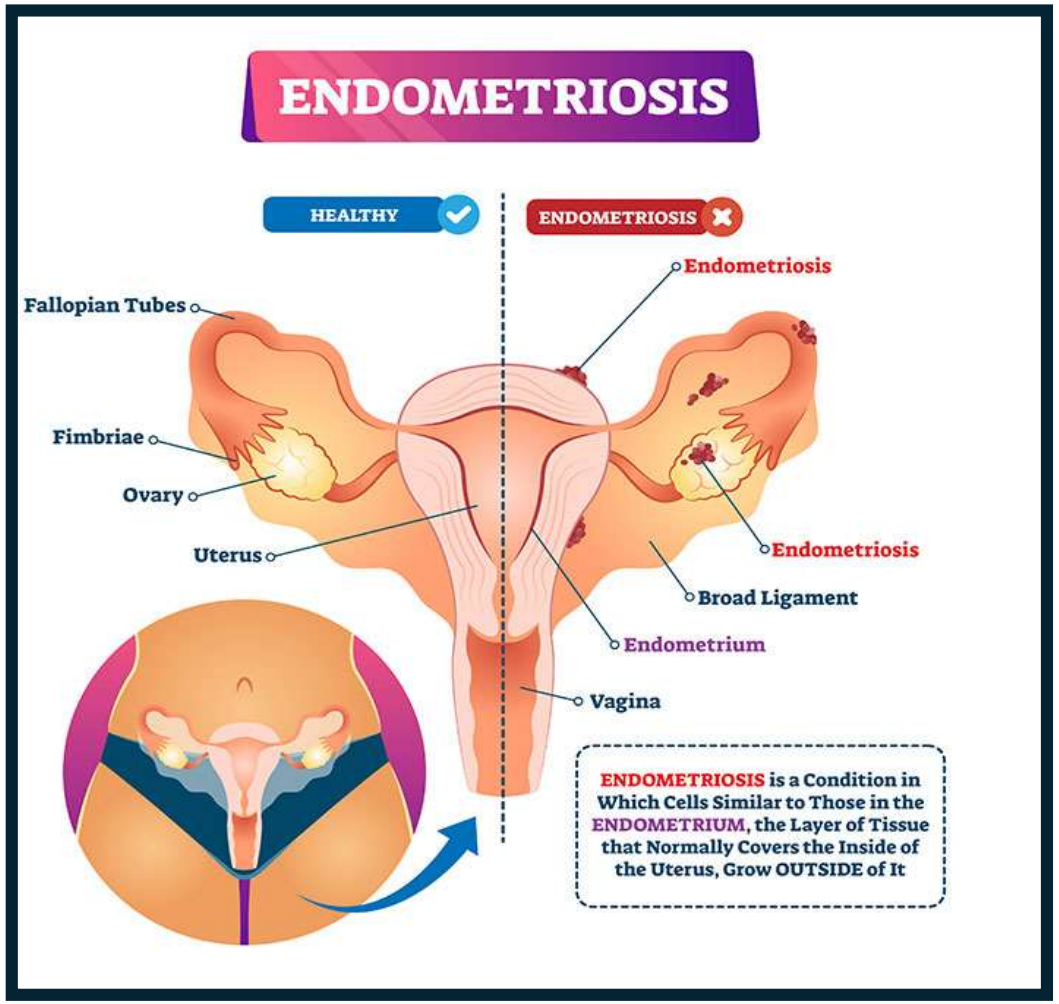
Endometriosis is a chronic disorder that affects women of reproductive age.

It is a public health concern due to a lack of proper diagnostic measures.

My talk outlines biomarkers that may aid in the early diagnosis of endometriosis when used independently or as panel of bio markers

These biomarkers may also be used for targeted treatment.

Endometriosis: Definition



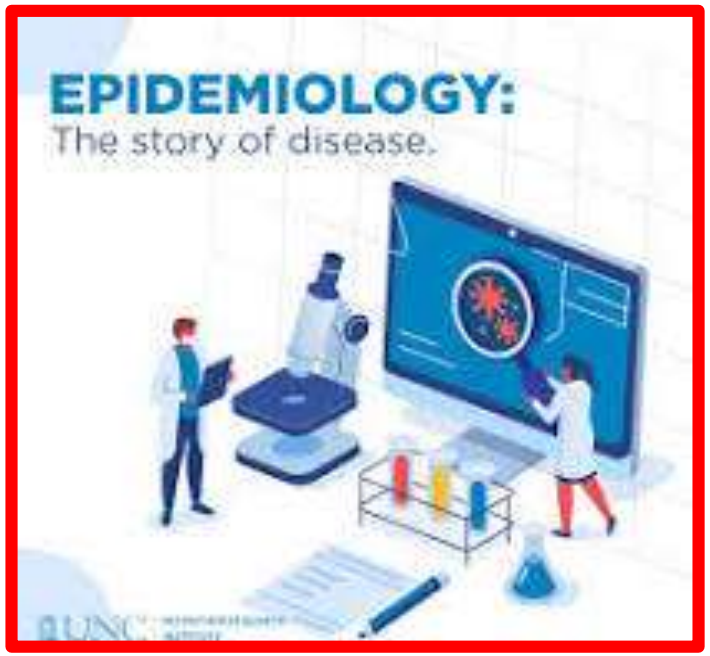
Presence of normal endometrial tissue abnormally implanted in locations other than uterine cavity

Implants grow & invade tissue in their vicinity, causing inflammatory reactions

Davila GW, et al. Medscape. Apr 25, 2016

Epidemiology

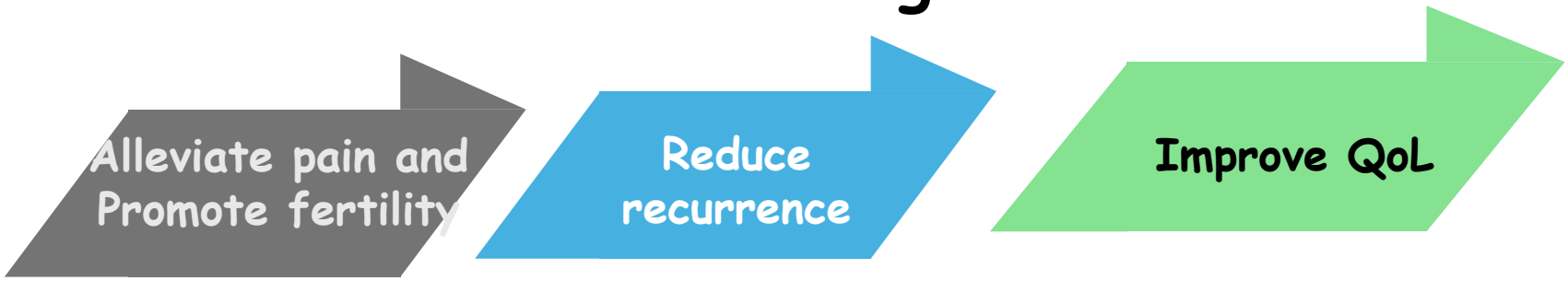
- Affects approximately 10-15% of women of reproductive age.
- More prevalent in women with a family history of endometriosis.
- Often diagnosed in the 3rd to 4th decade of life, though symptoms can begin earlier.
- Increased risk associated with early menarche, shorter menstrual cycles, and prolonged exposure to estrogen.



American Society for Reproductive Medicine [ASRM]

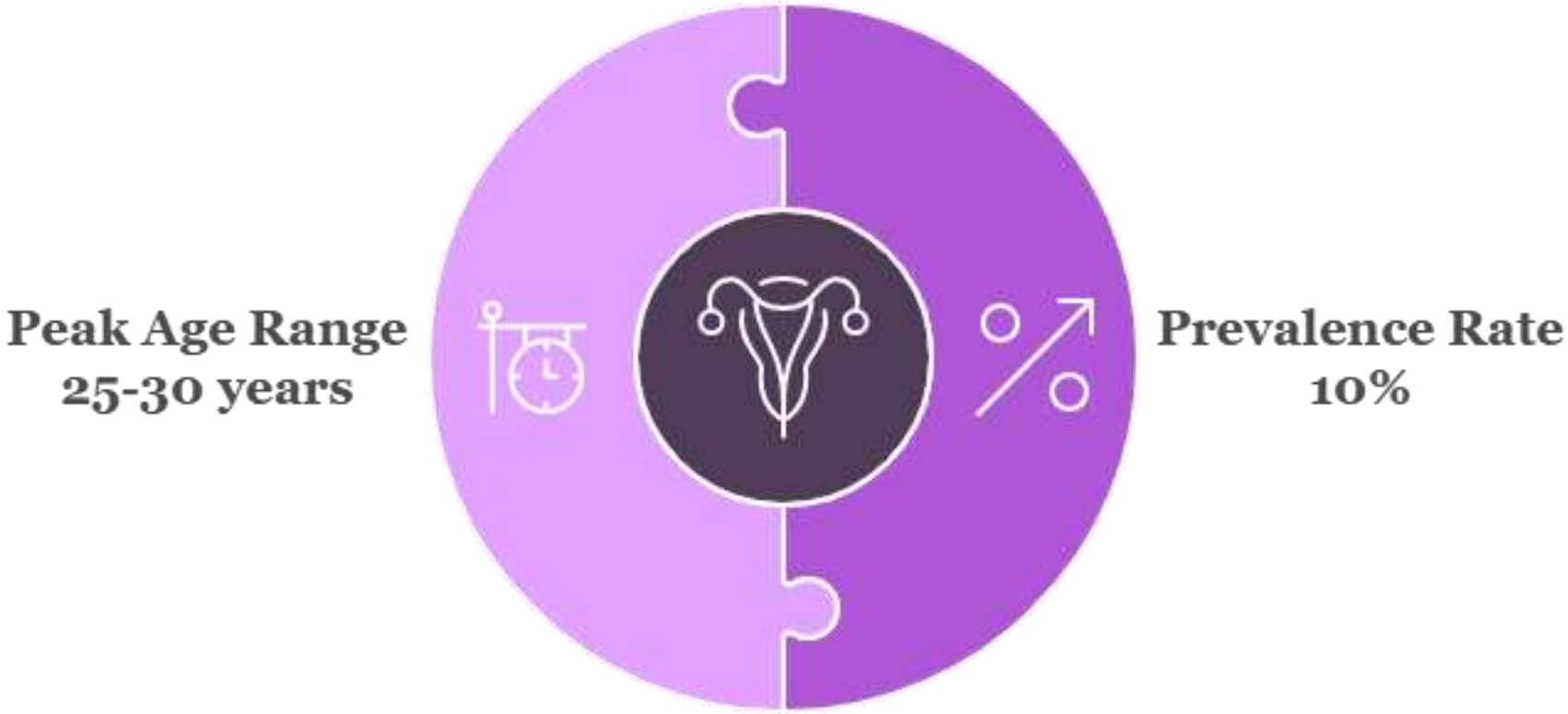
"Endometriosis should be viewed as a chronic inflammatory disease that requires a life-long management plan with the goal of maximizing the use of medical treatment and avoiding repeated surgical procedures"

Treatment goals



Prevalence

Endometriosis is a public health disorder affecting ~ 247 million women globally and ~ 42 million women in India.



Gajbhiye RK. Endometriosis and inflammatory immune responses: Indian experience. Am J Reprod Immunol. 2023 Feb;89(2):e13590

Endometriosis - Prevalence

Endometriosis is a highly prevalent condition!

Younger age at onset predicts more severe the disease!

25%–40% of infertile women

75% of women with chronic pelvic pain

40%–60% of women with dysmenorrhea

1. Ballweg ML et al. J Pediatr Adolesc Gynecol 2003; *2. Child TJ et al. Drugs 2001;*
3. Cramer DW et al. Ann N Y Acad Sci 2002; *4.WHO statement 2019 .*

Economic Burden

What are the costs of Endometriosis?

In one year, on average a women with endometriosis will incur:

$2,898 in direct costs

$12,330 in indirect costs

50% women have >17.6 days absenteeism /year

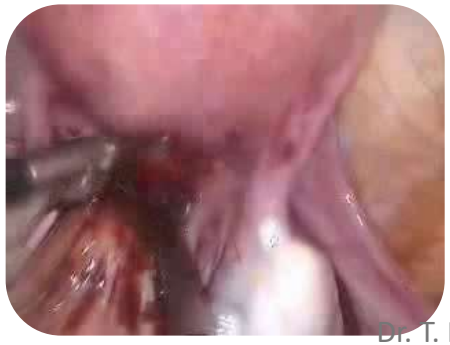
Kiejoff et al, 1987

Ovarian Endometriosis

Nodules implant in the lining of ovaries. When tissue around these areas hardens it can develop and proliferate into the fallopian tubes and bowels [4].

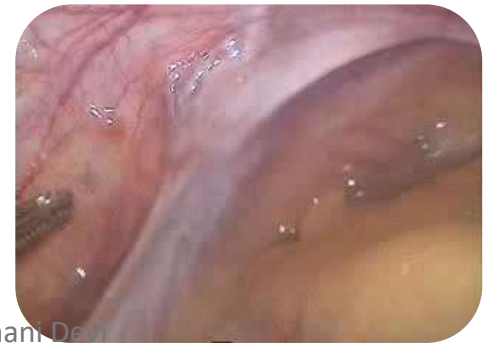
Deep Infiltrating Endometriosis

The nodules implant at least 5mm below the peritoneum [5]. Structures penetrated can include the uterosacral ligaments, bowel, bladder and ureters [4].



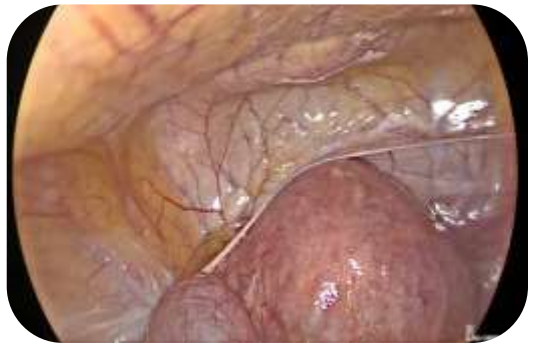
Peritoneal Endometriosis

The peritoneum is the lining of the abdomen. Peritoneal endometriosis occurs when endometrial cells travel to and implant in the peritoneal wall.

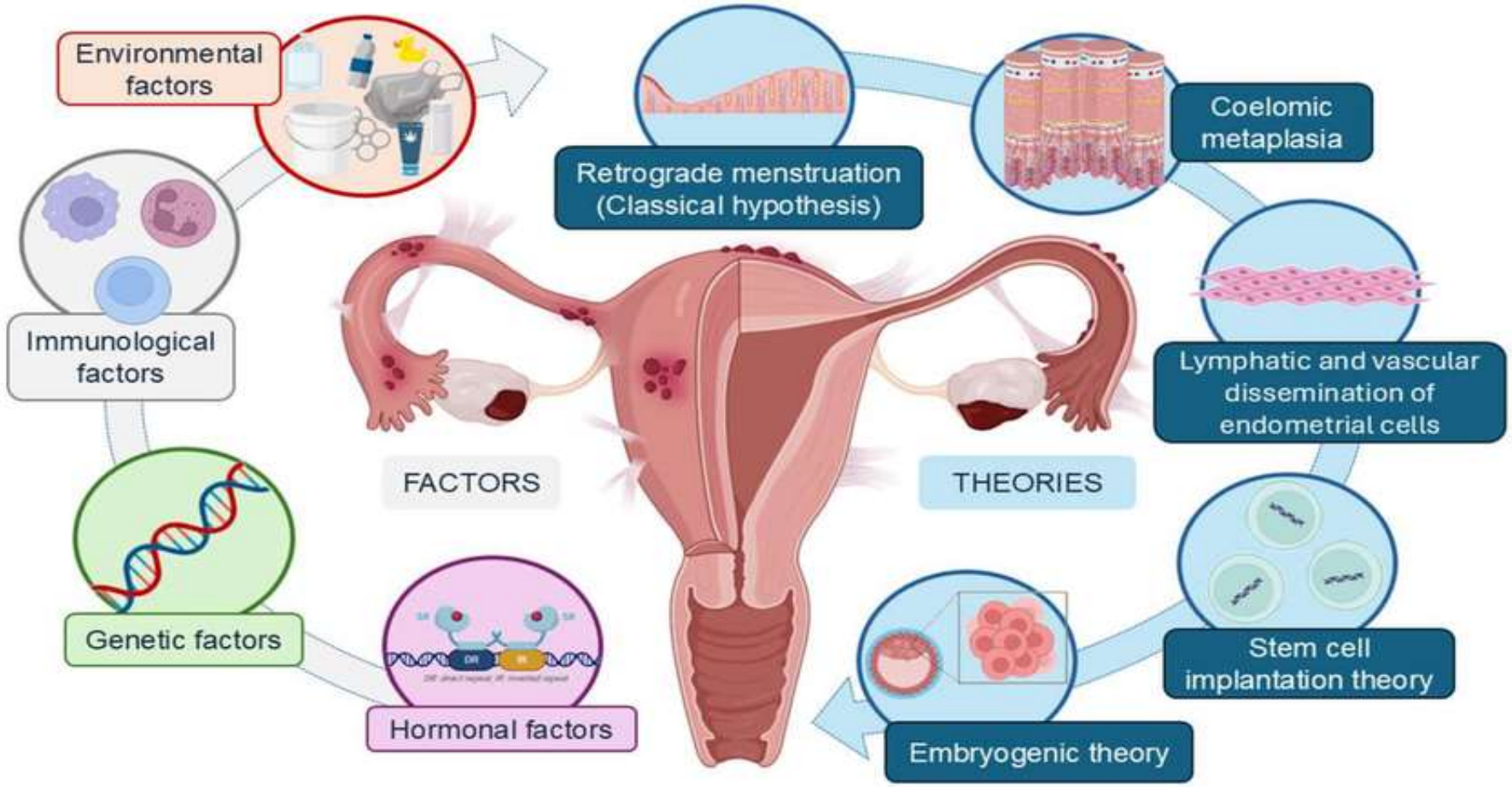


Adenomyosis

Presence of ectopic endometrial tissue within the myometrium which responds to cyclical hormonal changes PRE

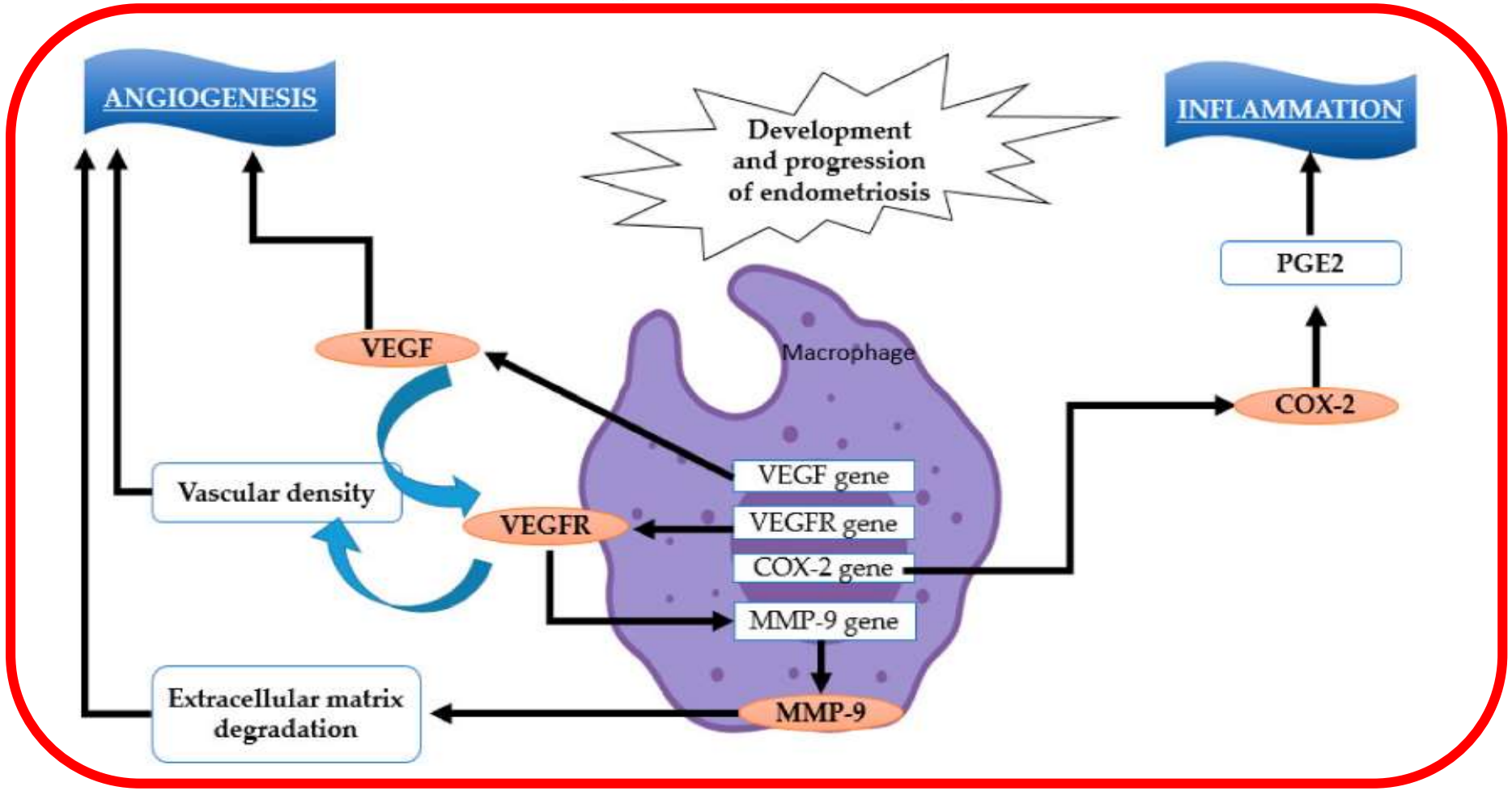


Theories on Pathogenesis of Endometriosis

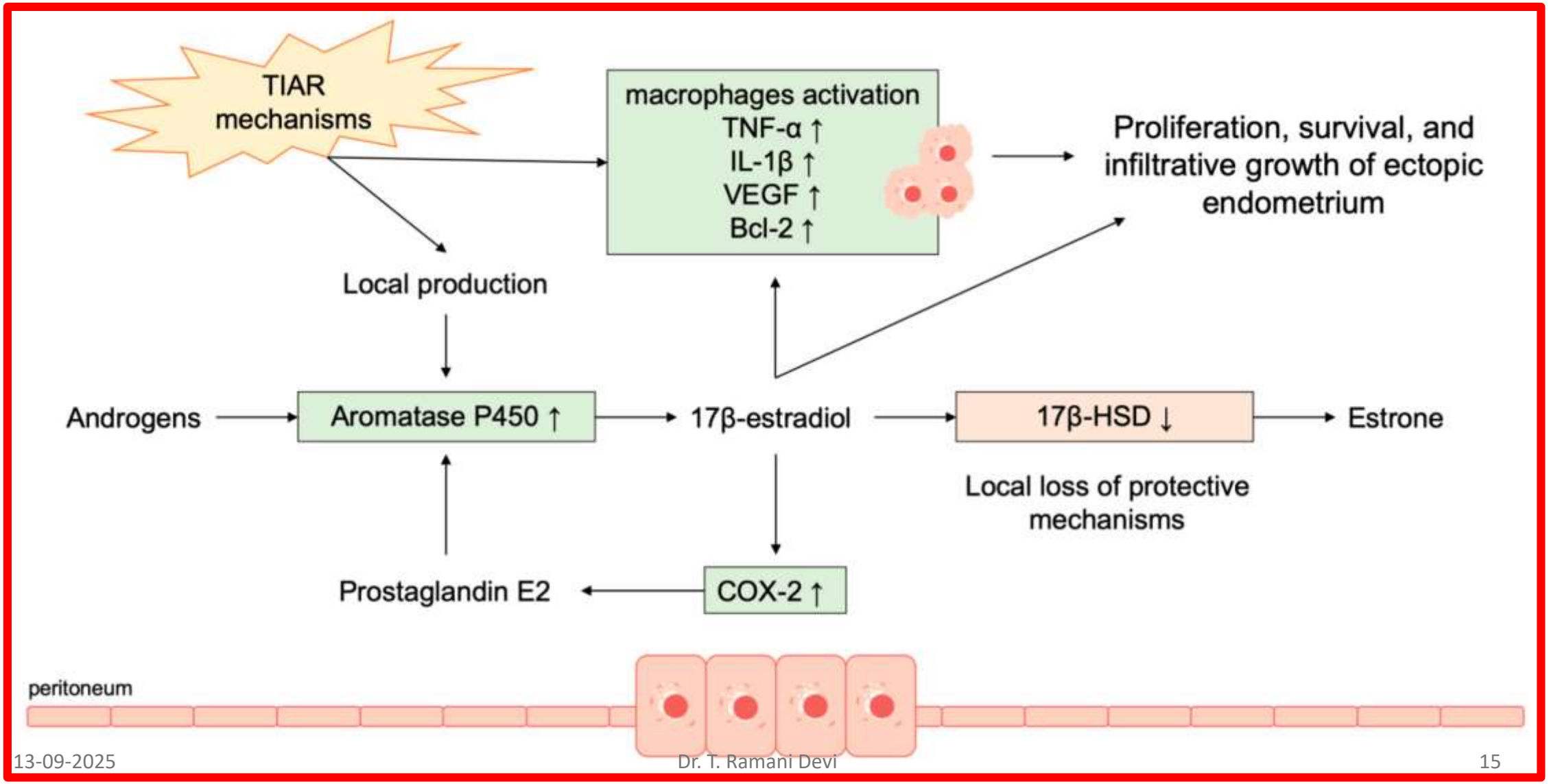


Macer ML, et al. Obstet Gynecol Clin. 2012;39(4):535-49. 2. Rafique S, et al. Clin Obstet Gynecol. 2017:60(3):485.

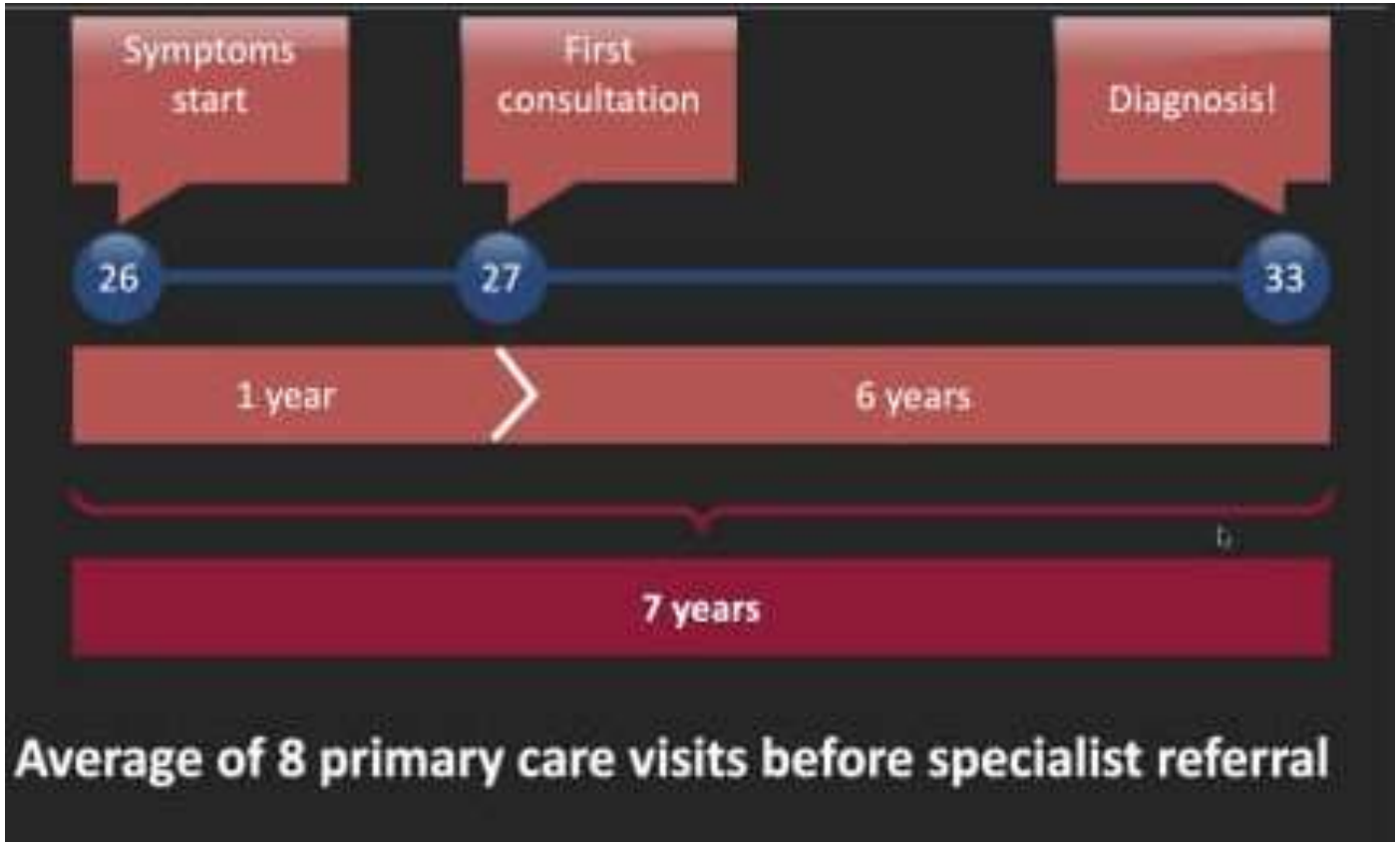
Angiogenic and inflammatory pathways for origin of endometriosis

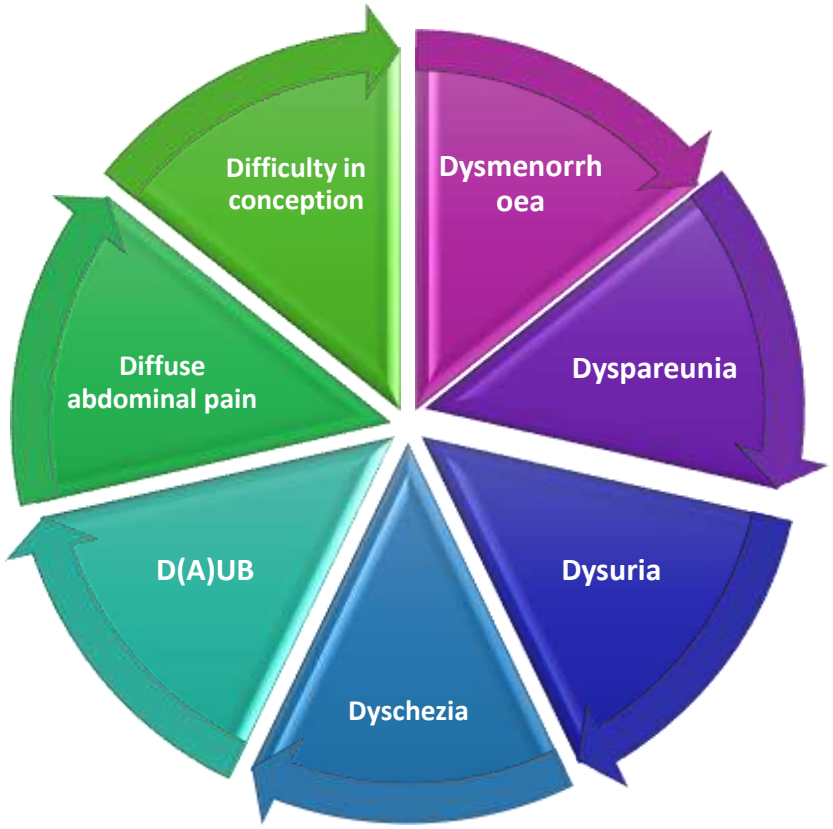


Key role of estrogens in the pathogenesis of endometriosis



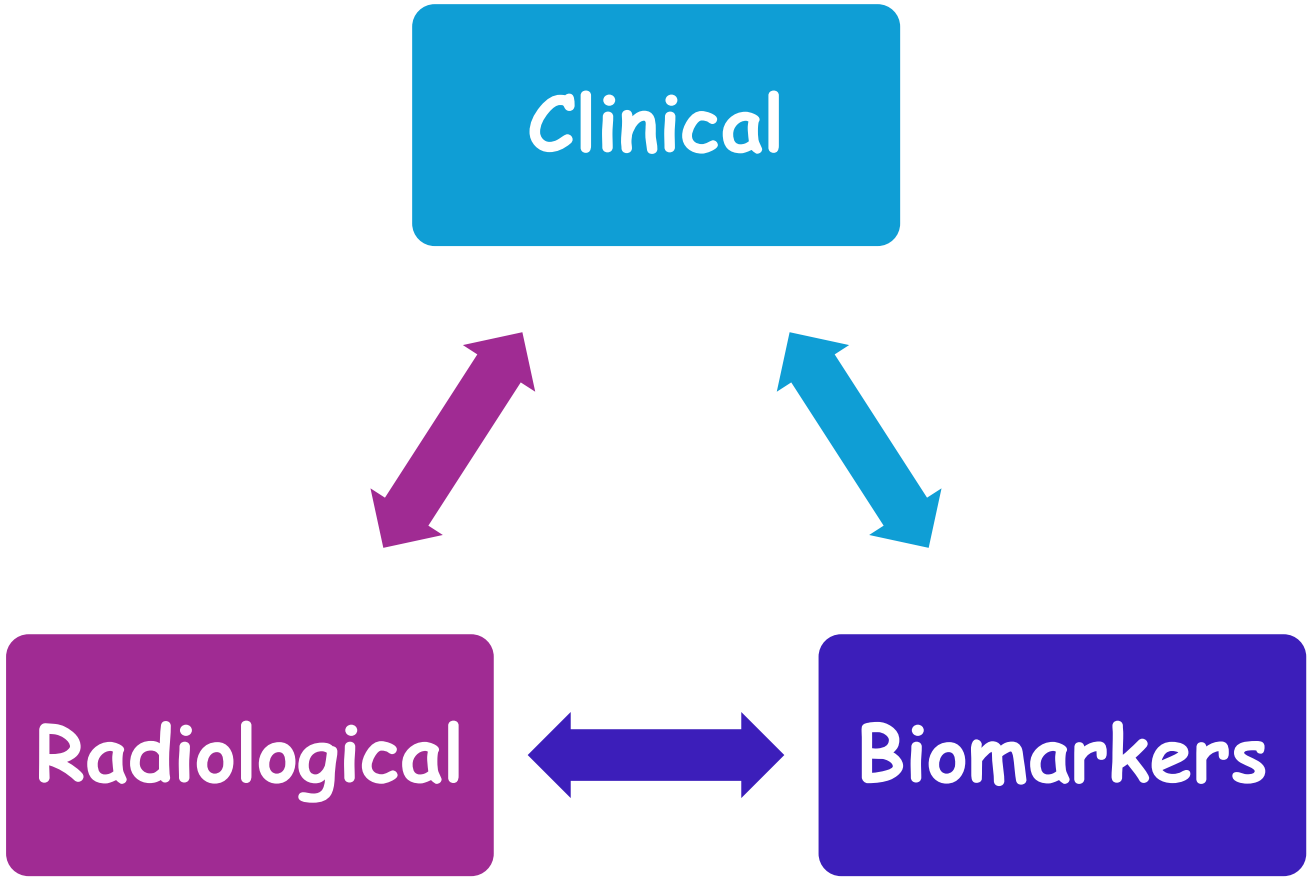
Sites & symptoms commonly affected in Endometriosis





Fatigue & depression

Endometriosis - non-surgical diagnosis



Endometriosis: How to optimize the diagnosis?

- Necessary to change the paradigm
- In 2019, there was NO PLACE for diagnostic laparoscopy when endometriosis was clinically suspected

Chapron et al 2019

Treatment with or without histology?

- Numerous learned societies have made recommendations that medical treatment can be prescribed for endometriosis without prior histological confirmation.

Rethinking mechanisms, diagnosis and management of endometriosis Charles Chapron1,2,3, Louis Marcellin1,2,3,*

Bruno Borghese1,2,3 and Pietro SantulliLeyland, N., Casper, R., Laberge, P., Singh, S. S. & SOGC. Endometriosis: diagnosis and management. J. Obstet. Gynaecol. Can. 32, S1–S32 (2010). 215.

American College of Obstetricians and Gynecologists. ACOG: Practice bulletin no. 114: Management of endometriosis. Obstet. Gynecol. 116, 223–236 (2010). 216.

Johnson, N. P. & Hummelshoj, L. World Endometriosis Society Montpellier, C. Consensus on current management of endometriosis. Hum. Reprod. 28, 1552–1568 (2013). 217.

Dunselman, G. A. et al. ESHRE guideline: management of women with endometriosis. Hum. Reprod. 29,

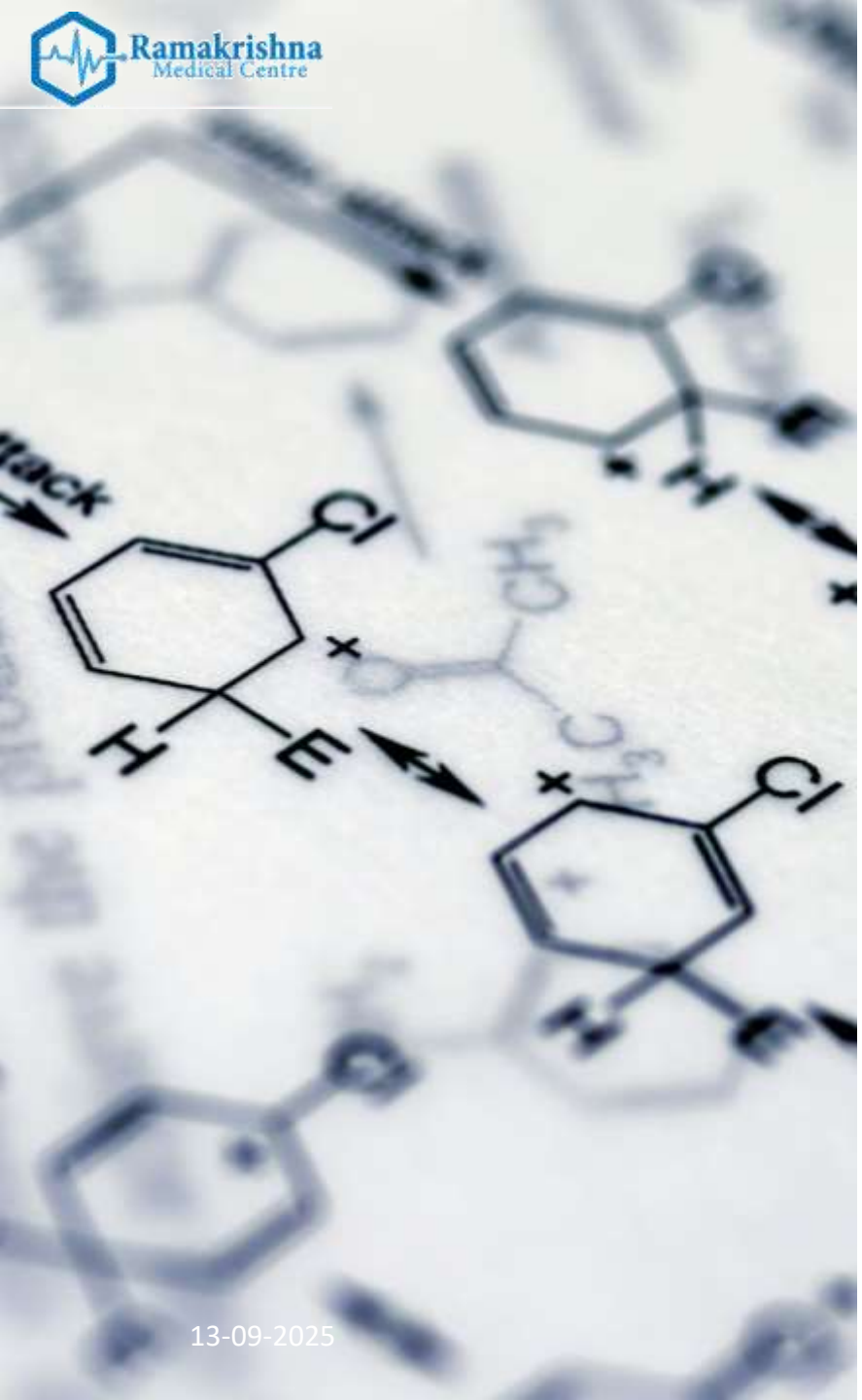
Role of biomarkers

- Biomarkers would have a clear impact in reducing time to diagnosis and monitoring the progress of the disease and the effectiveness of treatment

- To replace invasive diagnostic methods, biomarkers could be considering clinically useful if they comply with predetermined criteria - sensitivity 94% and specificity 79%

May, K.E.; Conduit-Hulbert, S.A.; Villar, J.; Kirtley, S.; Kennedy, S.H.; Becker, C.M. Peripheral biomarkers of endometriosis: a systematic review. Hum. Reprod. Update 2010, 16, 651-74.

Luisi, S.; Pinzauti, S.; Regini, C.; Petraglia, F. Serum markers for the noninvasive diagnosis of endometriosis. Womens Health (Lond.) 2015, 11, 603-610.



What is a bio marker?

- A biomarker is a biological molecule that can be "objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention"

- Therefore, a biomarker or a panel of biomarkers could be an expedient diagnostic tool

Non invasive diagnostic markers

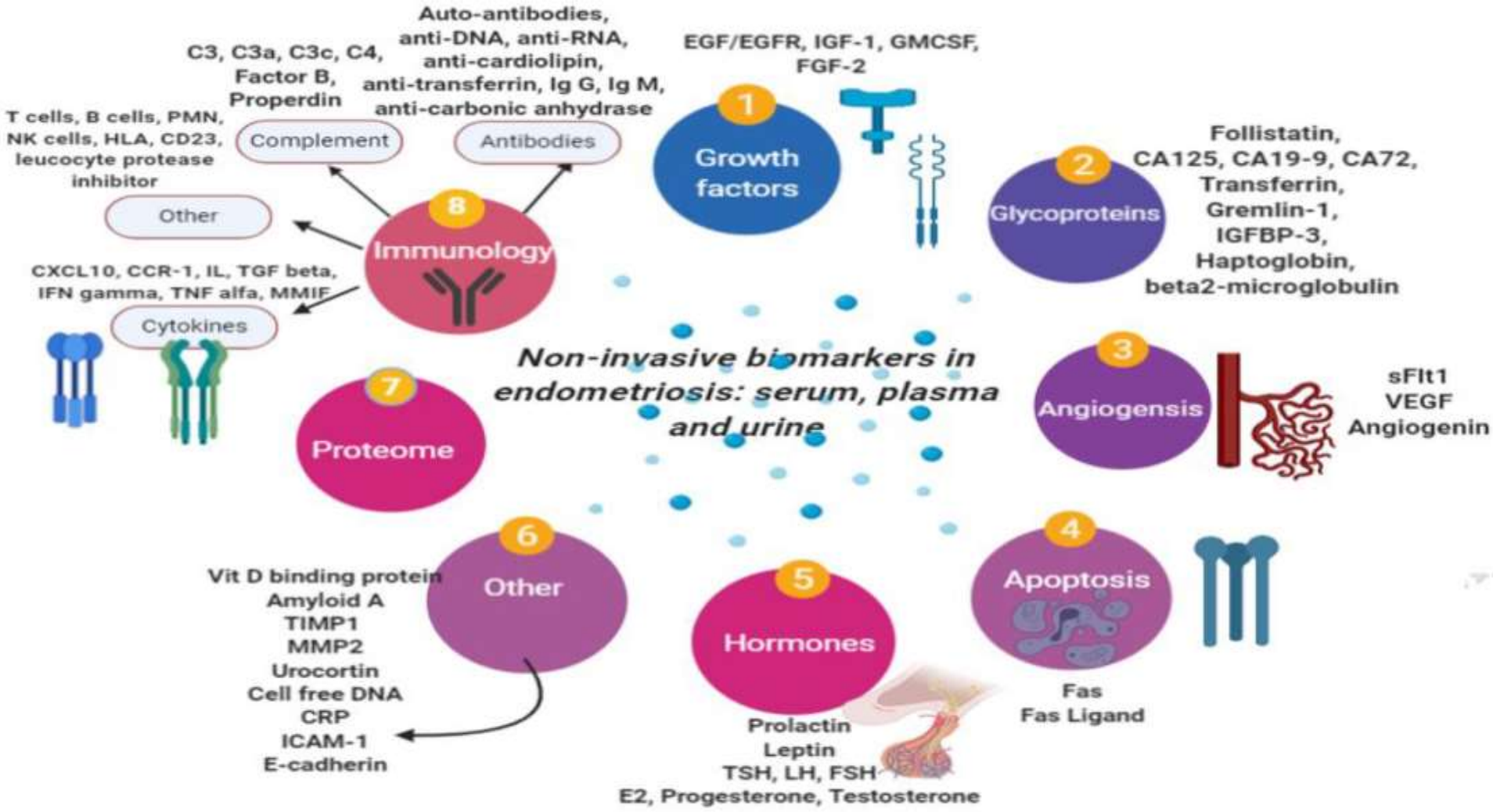
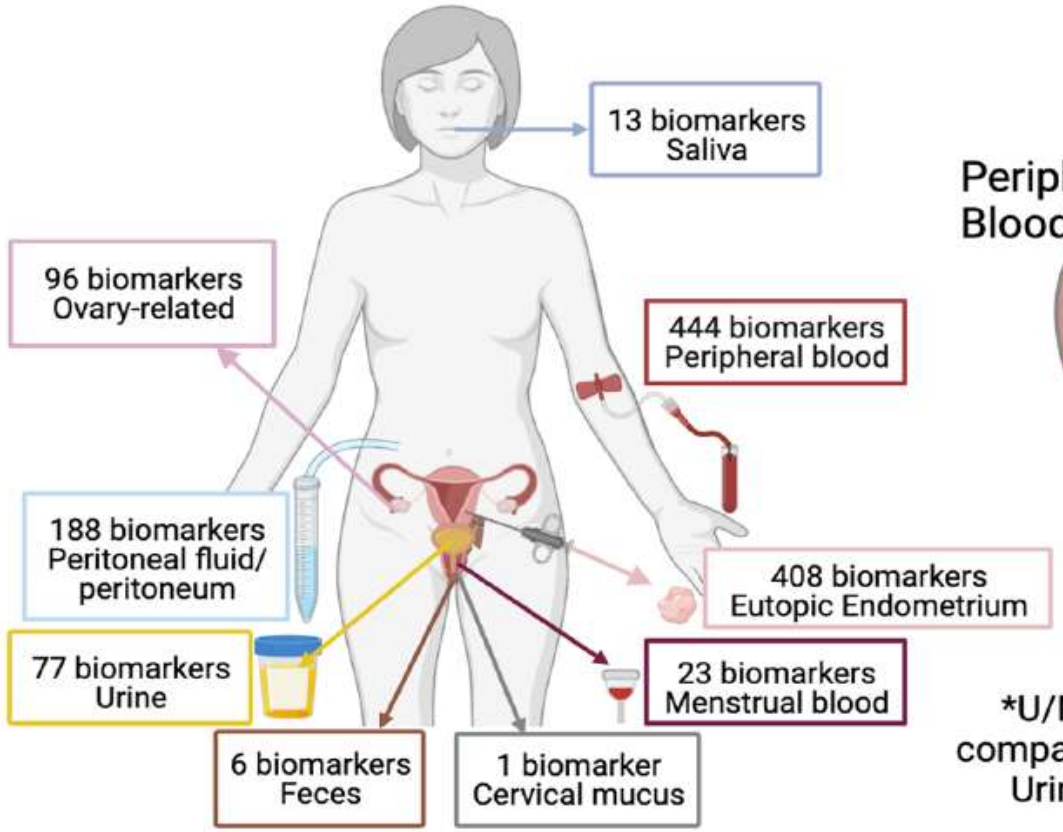
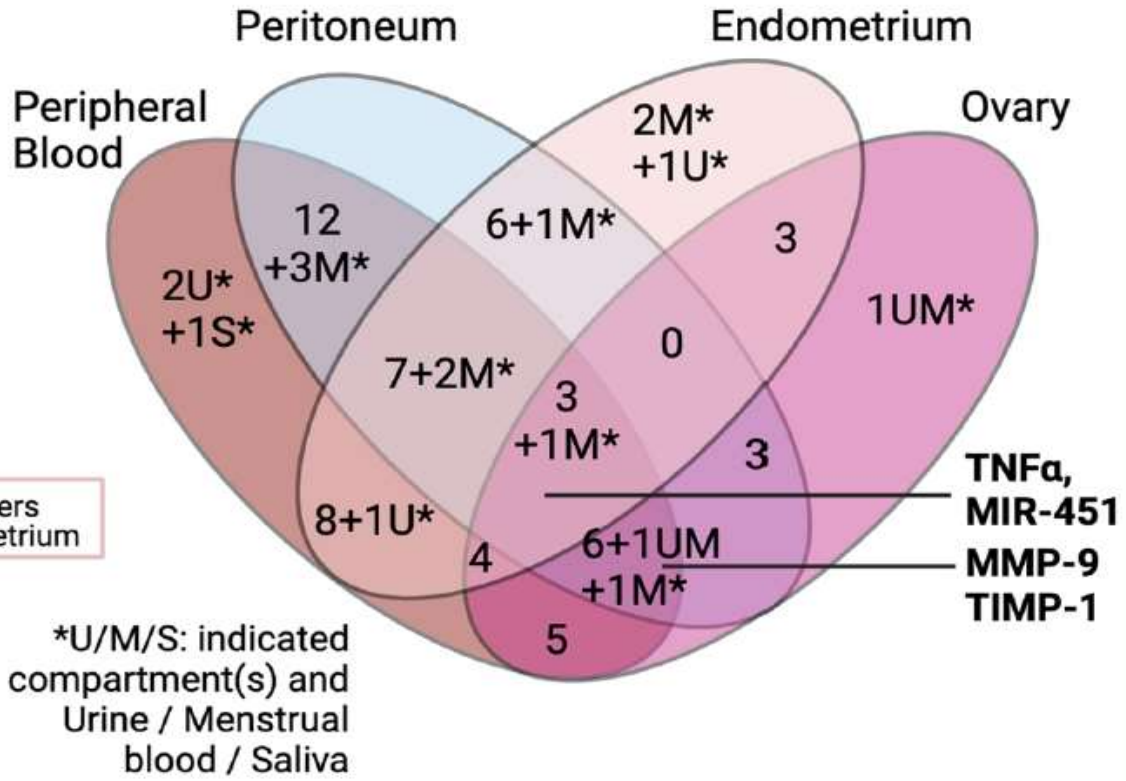


Figure 1. Putative noninvasive biomarkers for endometriosis [12].

Keywords Endometriosis, Candidate biomarkers, Biological compartments, Endometriosis phenotypes

Graphical Abstract

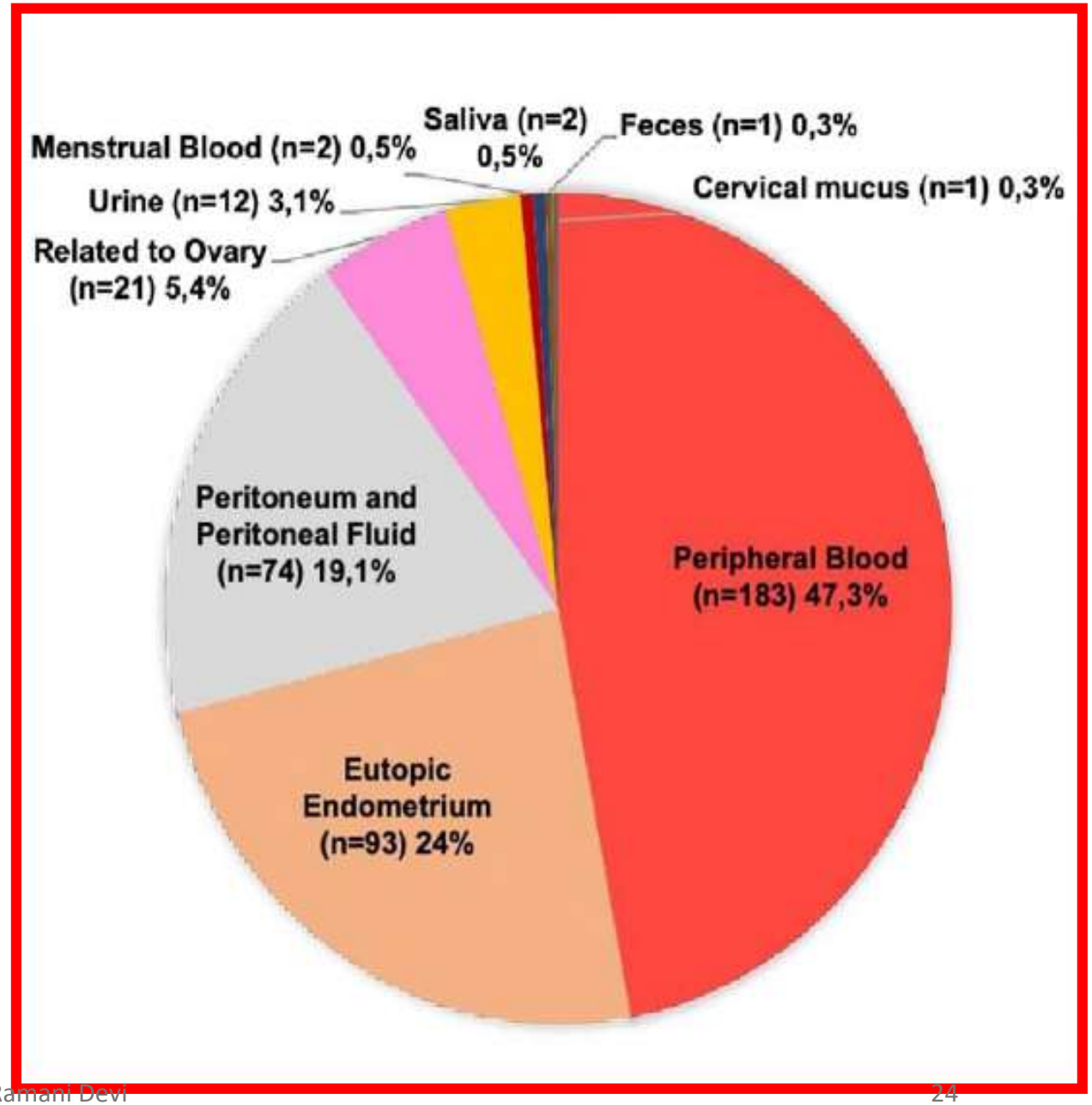




Numerous biomarkers found in different biological compartments from 447 studies comparing women with versus without endometriosis

Limited overlap of the independently validated biomarkers in the different biological compartments

Biological Compartment Analysis



Biomarkers in Endometriosis

- CA-125
- CA-19 9
- Follistatin
- Urocortin
- Activin-A
- Oxidative stress
- Macrophages
- NK Cells
- Microbiome

- Interleukins & TNF-Alpha
- Circulating endometrial cells
- Neutrophil/lymphocytic ratio
- Integrins-cell adhesive molecules
- Immunological markers
- HE-4
- Micro RNAs

Glycoproteins- (CA-125)

It is a glycoprotein of the ovarian epithelial cells, as a potential marker for endometriosis

Cut off level is 35 U/ml; Positive results of CA-125 in the middle of the menstrual cycle indicate very high risk of endometriosis

The sensitivity of endometriosis stage III and IV was 63.1%, compared to only 24.8% in stage I and II.

CA-125 remains the only marker widely used in clinical practice as prognostic rather than diagnostic marker.

Oliveira, M.A.P.; Raymundo, T.S.; Soares, L.C.; Pereira, T.R.D.; Demôro A.V.E.How to Use CA-125 More Effectively in the Diagnosis of Deep Endometriosis. Biomed. Res. Int. 2017,,

CA 19-9

Endometrium also produces CA 19-9, researchers began to look for its application in diagnosing endometriosis.

Comparing to CA-125 its specificity and sensitivity are respectively 86-89% and 52- 61%.

Other glycoproteins that were taken into consideration in the studies were CA 15-3, CA 72-4, α-fetoprotein (AFP) and carcinoembryonic antigen (CEA).

Activin A

- Activin is a growth factor belonging to TGF-β family.
- It is produced by a healthy endometrium and it peaks in the secretory phase
- It plays a role in the immunological processes involved in the pathogenesis of endometriosis.
- In endometriosis level increases both in eutopic and ectopic endometrium.
- The highest levels was observed in ovarian endometrioma (OMA)

.

Reis, F.M.; Luisi, S.; Abrão, M.S.; Rocha, A.L.; Viganò, P.; Rezende, C.P.; Florio, P.; Petraglia, F. Diagnostic value of serum activin A and follistatin levels in women with peritoneal, ovarian and deep infiltrating endometriosis. Hum. Reprod. 2012, 27, 1445-1450.

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Follistatin

The highest plasma follistatin level was observed in the OMA and peritoneal forms in relation to deep infiltrating endometriosis (DIE) and healthy controls, which excludes its use as a marker of endometriosis

The combination of activin A and follistatin as markers of endometriosis showed the highest effectiveness.

Rocha, A.L.; Carrarelli, P.; Novembri, R.; Sabbioni, L.; Luisi, S.; Reis, F.M.; Petraglia, F. Altered expression of activin, cripto, and follistatin in the endometrium of women with endometrioma. Fertil. Steril. 2011, 95, 2241-2246.

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Growth factors & peptides- Urocortin

Urocortin is produced by eutopic and ectopic endometrium.

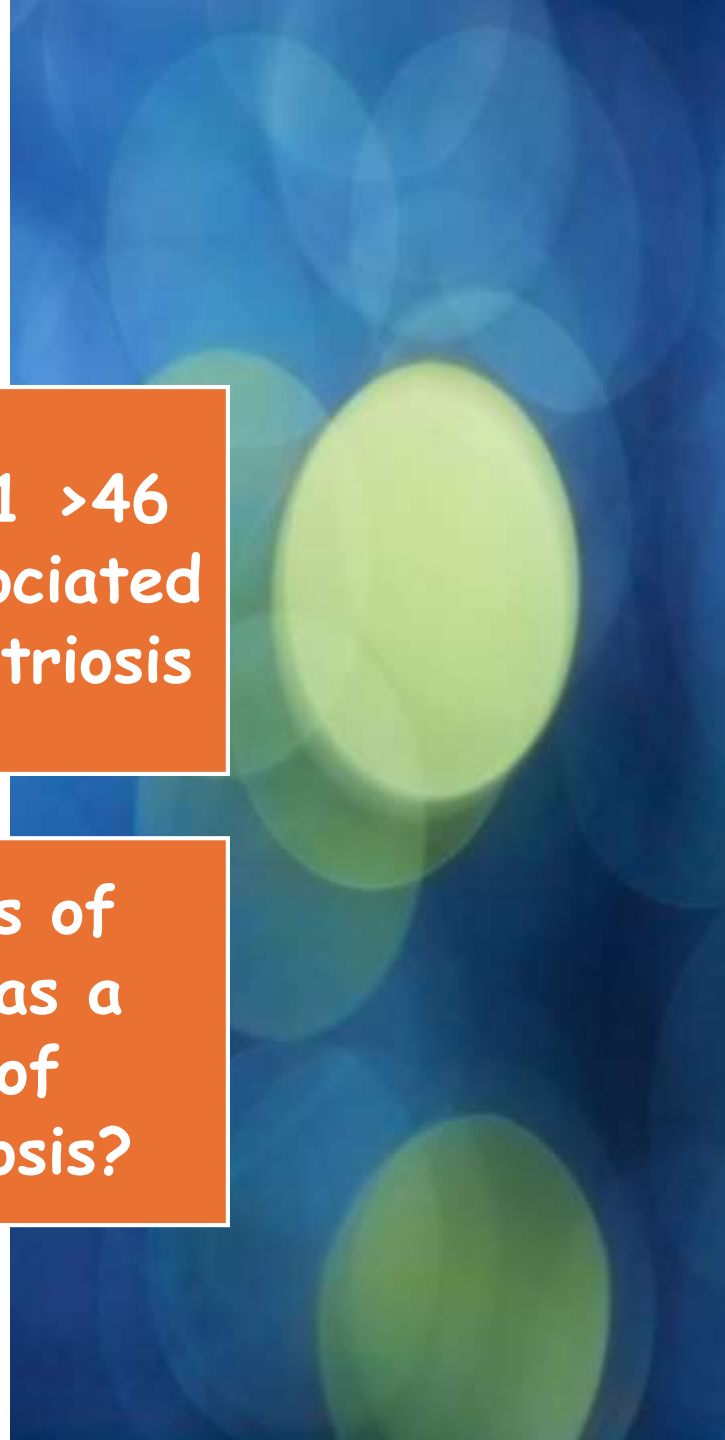
Urocortin levels have high sensitivity

Plasma Ucn1 >46 pg/ml is associated with endometriosis

It is increased in women who had both infertility and chronic pelvic pain.

Urocortin has 88% sensitivity and 90% specificity, better than CA-125

Usefulness of urocortin as a marker of endometriosis?



Oxidative stress

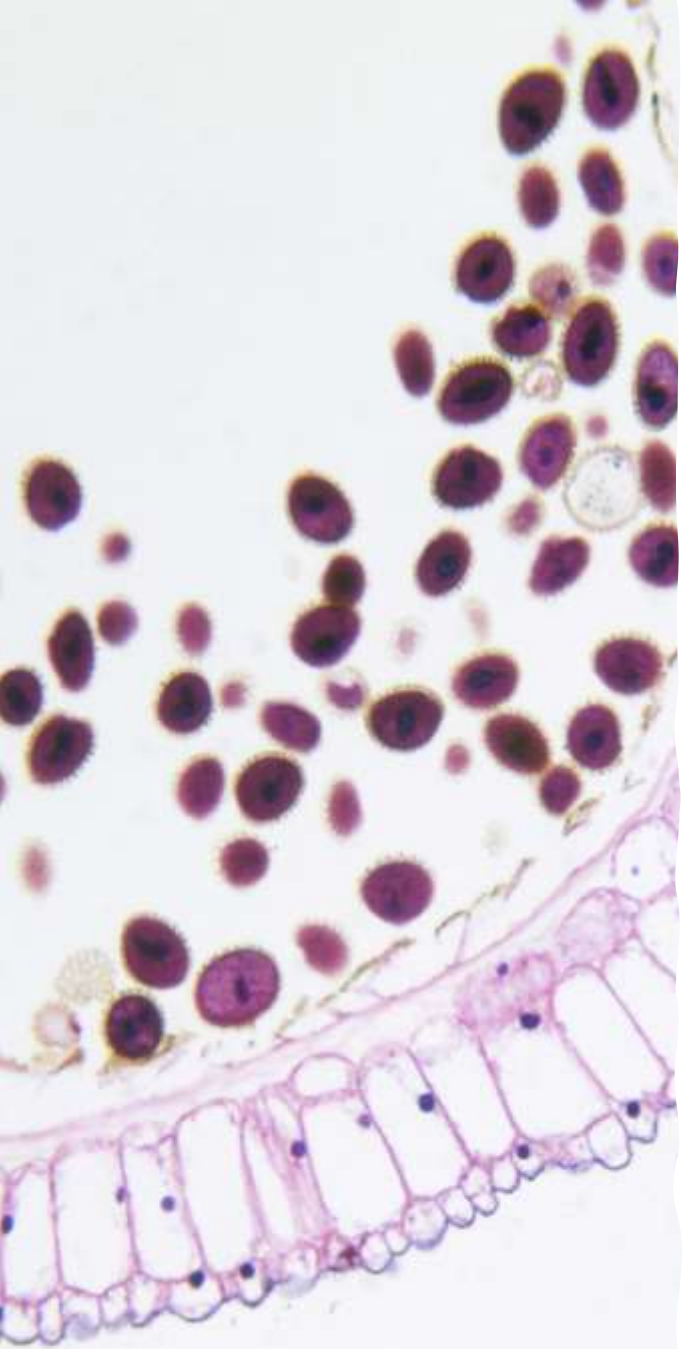
Imbalance between oxidants and anti-oxidants is called oxidative stress.

It has significant role in inflammatory response of endometriosis

ROS associated with endometriosis and subfertility.

There is possible use of ROS markers as diagnostic tests for endometriosis

Thézénas, M.L.; De Leo, B.; Laux-Biehlmann, A.; Bafligil, C.; Elger, B.; Tapmeier, T.; Morten, K.; Rahmioglu, N.; Dakin, S.G.;Charles, P.; Martinez, F.E.; Steers, G.; Fischer, O.M.; Mueller, J. Amine oxidase 3 is a novel pro-inflammatory marker of oxidativestress in peritoneal endometriosis lesions. Sci. Rep. 2020, 10, 56-65.



Immunological markers

- Dysfunction of the immune system is involved in the pathogenesis of endometriosis.
- Macrophages are one of the cells found in significant amounts in the peritoneal fluid.
- They are responsible for ectopic endometrial cell adhesion, implantation and growth.

Aznaurova, Y.B.; Zhumataev, M.B.; Roberts, T.K.; Aliper, A.M.; Zhavoronkov, A.A. Molecular aspects of development and regulation of endometriosis. Reprod. Biol. Endocrino. 2014, 12, 50

Macrophages

- Macrophages are the source of VEGF responsible for angiogenesis in response to TNF-α & IL-6
- According to some study the level of TNF-α increased in patients with endometriosis and correlated with its severity
- It has been observed to significantly increase in endometrial lesions, in advanced stages

Lin, Y.J.; Lai, M.D.; Lei, H.Y.; Wing, L.Y., Xavier, P.; Belo, L.; Beires, J.; Rebelo, I.; Martinez-de-Oliveira

Interleukin and TNF alpha

IL-6 has a Sensitivity of 0.70 (95% CI 0.57–0.80) and a high specificity of 1.00 (95% CI 0.88–1.00) with a cutoff value of IL-6 >12.20 pg/mL

TNF alpha is >12.45 pg/mL in endometriosis

Few studies found inconsistent results in endometriosis

Future researchers focus on the diagnostic efficacy of IL-6 combined with other cytokines instead of IL-6 alone.

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Soluble intercellular-adhesion molecule-1

It is associated with reduced cytotoxic activity of NK cells.

It may be relevant to implantation disorders and the formation of endometrial lesions

Matalliotakis et al. demonstrated that the level of sICAM-1 was higher in women suffering from endometriosis infertility compared to healthy controls.

Elevated monocyte chemotactic protein-1 (MCP-1) values were observed in peritoneal fluid and plasma in women with endometriosis, especially in the early stages of the disease. (Luisi, S etal)

Neutrophil/Lymphocyte ratio

Neutrophil/lymphocyte ratio can be applied as a diagnostic method for endometriosis

They have shown that women with endometriosis may have neutrophilia coexisting with lymphocytopenia.

The combined use of neutrophil/lymphocyte ratio and CA-125 concentration demonstrated high sensitivity for endometriosis detection with sensitivity of 69.3% and specificity of 83.9%

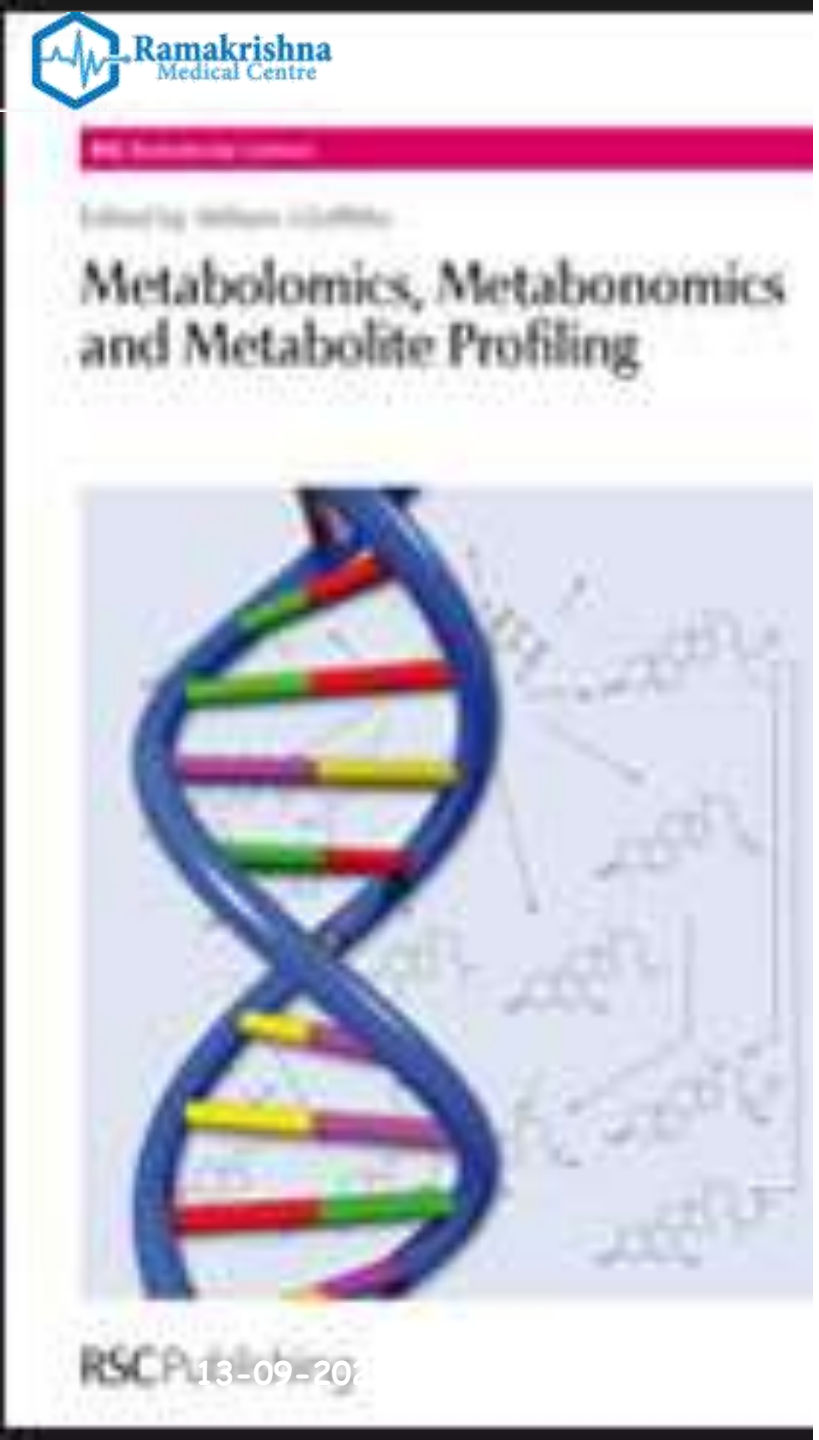
Cho, S.; Cho, H.; Nam, A.; Kim, H.Y.; Choi, Y.S.; Park, K.H.; Cho, D.J.; Lee, B.S. Neutrophil-to-lymphocyte ratio as an adjunct to CA-125 for the diagnosis of endometriosis. Fertil. Steril. 2008, 90, 2073-2079.

J Clin Med. 2019 Nov 11;8(11):1938. doi: 10.3390/jcm8111938

Circulating Endometrial Cells: A New Source of Information on Endometriosis Dynamics

Eliska Pospisilova [1,2,†], Imrich Kiss [1,2,3,†], Helena Souckova [2], Pavel Tomes [3], Jan Spicka [1], Rafal Matkowski [4,5], Marcin Jedryka [4,5], Simone Ferrero [6], Vladimir Bobek [1,5,7,8,9], Katarina Kolostova [1,5,*]

- The focus of the presented work was to isolate and characterize circulating endometrial cells (CECs) enriched from peripheral blood (PB) of patients with diagnosed endometriosis.
- CEC assay had 89.5% sensitivity and 87.5% specificity in distinguishing endometriosis from other benign ovarian masses.
- The molecular characteristics of CECs could be supportive for an understanding of endometriosis pathogenesis and treatment decisions in the future.



Predictive biomarkers for early diagnosis

- Proteomics -Specific plasma biomarker obtained during menses identifies the protein finger-prints which are markers of the disease can be dysregulated
- Proteomic technologies along with genetic profiling are newer modalities of non-invasive diagnosis.
- Genetic marker : Saliva based diagnosis of genetic marker may replace surgical procedure for diagnosis.

Review article

Endometrial nerve fibres in endometriosis

Natsuko Tokushige Ph.D., Moamar al-Jefout M.D., Hilmy Salih M.D., Ian S Fraser M.D.

Department of Obstetrics and Gynaecology, Queen Elizabeth II Research Institute for Mothers and Infants, University of Sydney, Australia.

Received: 15 May 2007;accepted: 5 August 2007

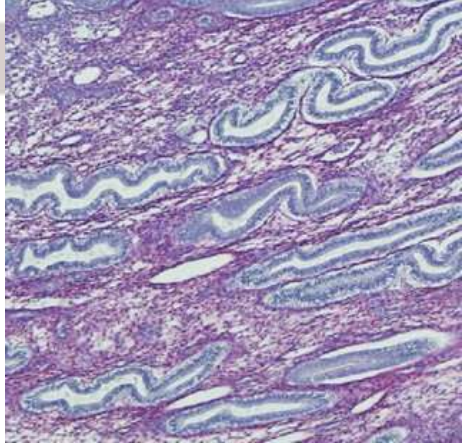
- Unmyelinated sensory nerve fibres (using the pan-neuronal marker PGP9.5) in the functional layer of endometrium in women with endometriosis and a significantly increased nerve fibre density in endometrium and myometrium in women with endometriosis compared to women without endometriosis.

- Sensory C nerve fibres were only detected in the functional layer of endometrium of women with endometriosis and never in women without endometriosis.

human reproduction ORIGINAL ARTICLE Gynaecology

Diagnosis of endometriosis by detection of nerve fibres in an endometrial biopsy: a double blind study

M. Al-Jefout[1,2,3,4], G. Dezarnaulds[2], M. Cooper[2], N. Tokushige[1],



- RESULTS: In women with laparoscopic diagnosis of endometriosis (n 64) the mean nerve fibre density in the functional layer of the endometrial biopsy was 2.7 nerve fibres per mm2 (+3.5 SD)..
- 6 women had endometrial nerve fibres but no active endometriosis seen at laparoscopy. The specificity and sensitivity were 83 and 98%,respectively, positive predictive value was 91% and negative predictive value was 96%
- Women with endometriosis and pain symptoms had significantly higher nerve fibre density in comparison with women with infertility but no pain (2.3 and 0.8 nerve fibre per mm2, respectively, (P 0.005).

- CONCLUSIONS: Endometrial biopsy, with detection of nerve fibres, provided a reliability of diagnosis of endometriosis which is close to the accuracy of laparoscopic assessment by experienced Gynaecological laparoscopists

International Journal of Molecular Sciences

MDPI

Article

Altered Composition of Microbiota in Women with Ovarian Endometrioma: Microbiome Analyses of Extracellular Vesicles in the Peritoneal Fluid

Sa-Ra Lee [1,†], Jae-Chul Lee [1,†], Sung-Hoon Kim [1,*], Young-Sang Oh [1], Hee-Dong Chae [1], Hochan Seo [2], Chil-Sung Kang [2] and Tae-Seop Shin [2]

- Received: 29 March 2021 Accepted: 23 April 2021 Published: 27 April 2021Received: 29 March 2021 Accepted: 23 April 2021 Published: 27 April 2021
- **45 women with OMA and 45 control gp enrolled**
- **Bacterial genomic DNA was sequenced using next generation sequencing of the 16S rDNA V3–V4 regions.**
- **Acinetobacter, Pseudomonas, Streptococcus, and Enhydrobacter significantly increased**
- **Propionibacterium, Actinomyces, and Rothia significantly decreased in the endometriosis group compared with those in the control group ($p < 0.05$)**
- **These findings strongly suggest that microbiome composition is altered in the peritoneal environment in women with endometriosis.**

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stics (Basel). 2020 Mar; 10(3): 163.
Published online 2020 Mar 17. doi: 10.3390/diagnostics10030163
PMCID: PMC7151170
PMID: 32192080

Microbiome Profile of Deep Endometriosis Patients: Comparison of Vaginal Fluid, Endometrium and Lesion

Camila Hernandes,[1,*] Paola Silveira,[2] Aline Fernanda Rodrigues Sereia,[2] Ana Paula Christoff,[2] Helen Mendes,[1] Luiz Felipe Valter de Oliveira,[2] and Sergio Podgaec[1]

- Samples from endometrium. endo lesions and vaginal fluid taken
- DNA was extracted and the samples were analyzed to identify microbiome by high-throughput DNA sequencing of the 16S rRNA marker gene
- Amplicon sequencing showed deep endometriotic lesions seems to present different bacterial composition, less predominant of *Lactobacillus* and with more abundant *Alishewanella*, *Enterococcus* and *Pseudomonas* than the control groups

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Microbiome & Endometriosis

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doi:10.1093/hropen/hoab003

human reproduction open ORIGINAL ARTICLE

A short anogenital distance on MRI is a marker of endometriosis

A. Crestani [1,*], C. Abdel Wahab[2], A. Arfi[1], S. Ploteau[3], K. Kolanska[1], M. Breban[1], S. Bendifallah[1,4,5], C. Ferrier[1], and E. Darai[1,4,5,6]

- Endometriosis pts had shorter MRI-AGD especially MRI -AGD-AF.(anterior anal verge to posterior fourchette)

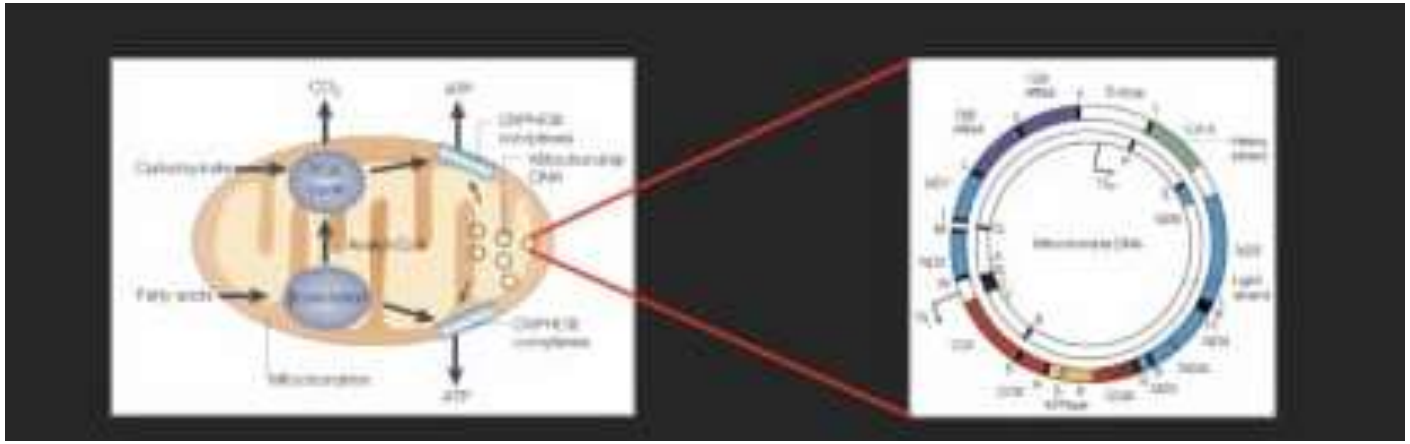
- As, MRI-AGD was independent of r-ASRM and Enzian classification, it can be used in diagnosing the early stage of disease

- Optimal MRI-AGD-AF cut off is 20mm

- Since, MRI has sensitivity of only 42% in diagnosing stage endometriosis – AGD measurement will be helpful for these patients

Figure 1. Measurements of anogenital distance. Anogenital...

Mitochondrial mutations as potential biomarkers?



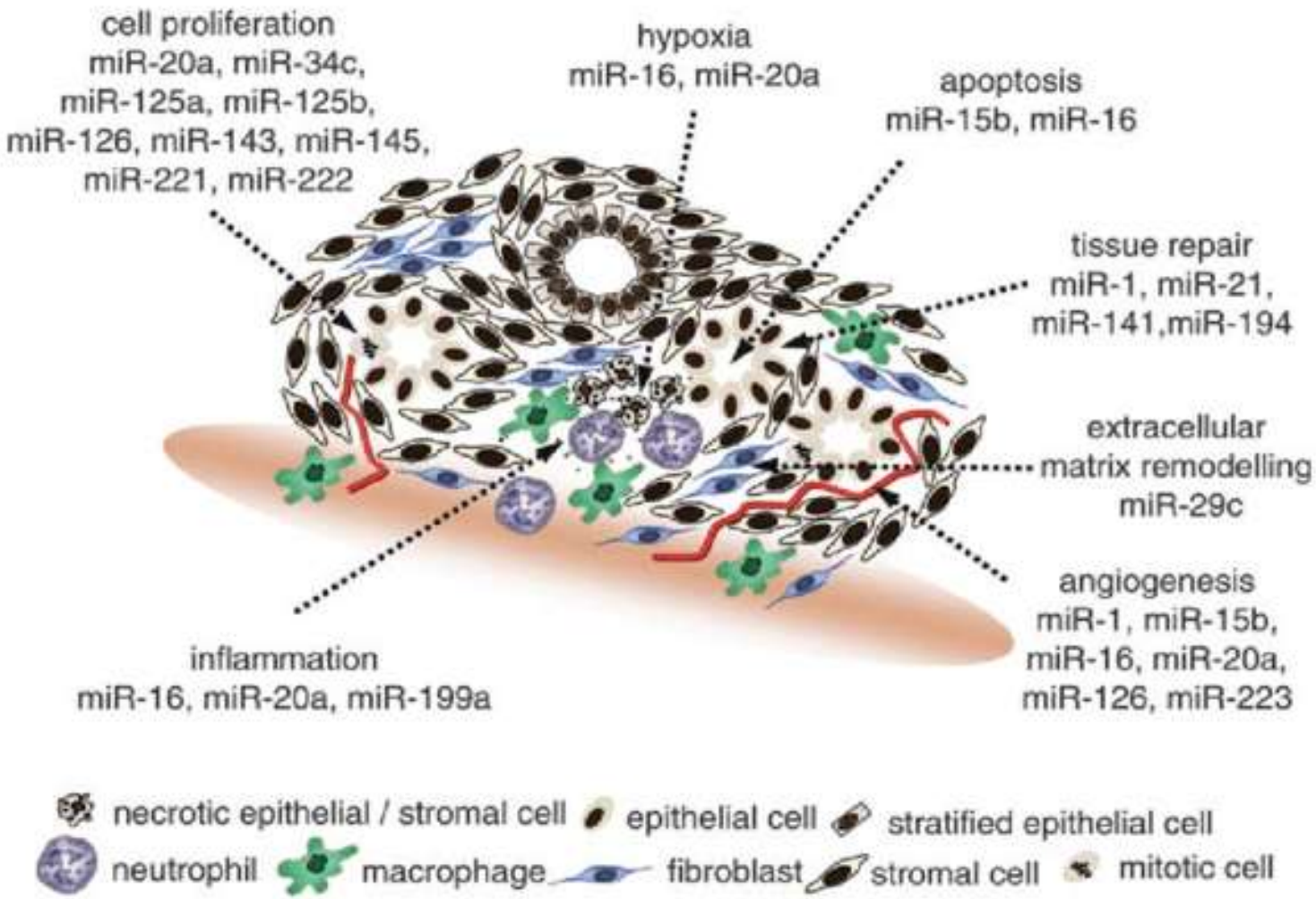
- Multiple mitochondria per cell
- Each mitochondrion contains multiple of copies of mitochondrial genome
- 16,569 bp containing 37 genes encoding 13 proteins, 22tRNAs, 2rRNAs
- Mutation rate about 100-fold higher than in nuclear genome

Taylor RW et al, Nat Rev Genet 2005

Nobel prize in medicine honors 2 scientists for their discovery of micro-RNA-2024

GARY RUVKUN & VICTOR AMBROS

mi RNA IN ENDOMETRIOSIS



What are mi- RNAs ?

- Total of 2500 mi RNAs have been detected in the human genome
- Alteration of miRNA occurs according to the disease of multiple organ systems.
- Each disease has their unique expression of miRNA.
- Aberrant expression of miRNAs are seen among endometriosis patients in the serum, plasma and saliva

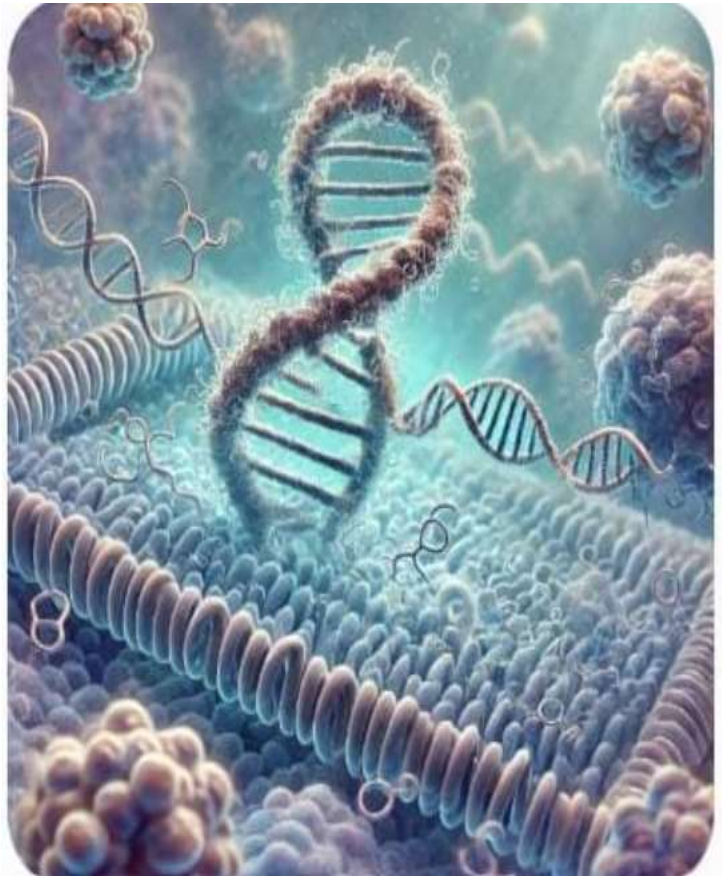
(Chakraborty et al., 2016; Srivastava et al., 2017).

What are mi- RNAs ?

- miRNAs are short nucleotide sequence of non-coding RNAs which are involved in the regulatory pathway
- They play a role in modulating genes involved in inflammation, angiogenesis, cell proliferation, oestrogen signalling and tissue remodelling
- They are single stranded RNA molecules of 21 – 25 nucleotides in length

(Bayraktar et al., 2017).

What are mi- RNAs ?



- miRNAs localize in RNA granules, endo membranes, and mitochondria, and secrete to outside cells via exosomes.
-
- These miRNAs and protected from the degradation by endogenous RNAase as they are found within the exosomes bound to protein complexes which makes them more stable and hence a better candidate marker

(Thomou et al., 2017).

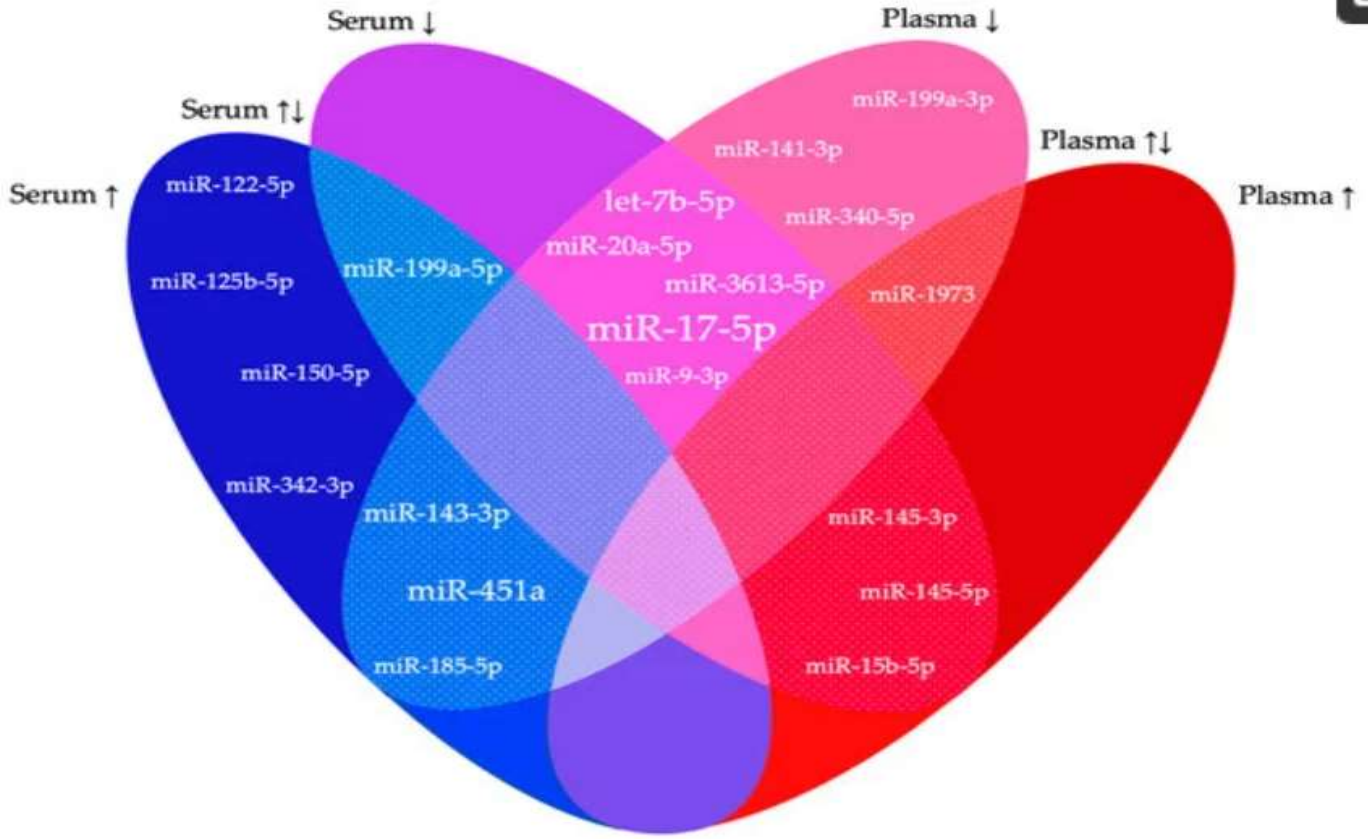
Role of mi-RNA in Endometriosis

- Recent study by Elahe et al, June 2020 showed plasma levels of novel miRNAs are differentially expressed in women with endometriosis compared to control group.
- This research work is an eye opener, to diagnose endometriosis using circulating bio-markers.
- Multiple miRNA panel is better than using a single miRNA to confirm the diagnosis

Identification of candidate microRNA markers of endometriosis with the use of next-generation sequencing and quantitative real-time polymerase chain reaction
Author links open overlay panel ElahePapariM.Sc.[a]MehrdadNoruziniaM.D., Ph.D.[a]LadanKashaniM.D.[b]Warren G.FosterPh.D.[c]

Circulating microRNAs as Non-Invasive Biomarkers in Endometriosis Diagnosis—A Systematic Review

by Arne Vanhie [1,*], Ellen Caron [2], Eveline Vermeersch [2], Dorien O [2], Carla Tomassetti [2], Christel Meuleman [2], Pieter Mestdagh [3,4] and Thomas M. D'Hooghe [2]

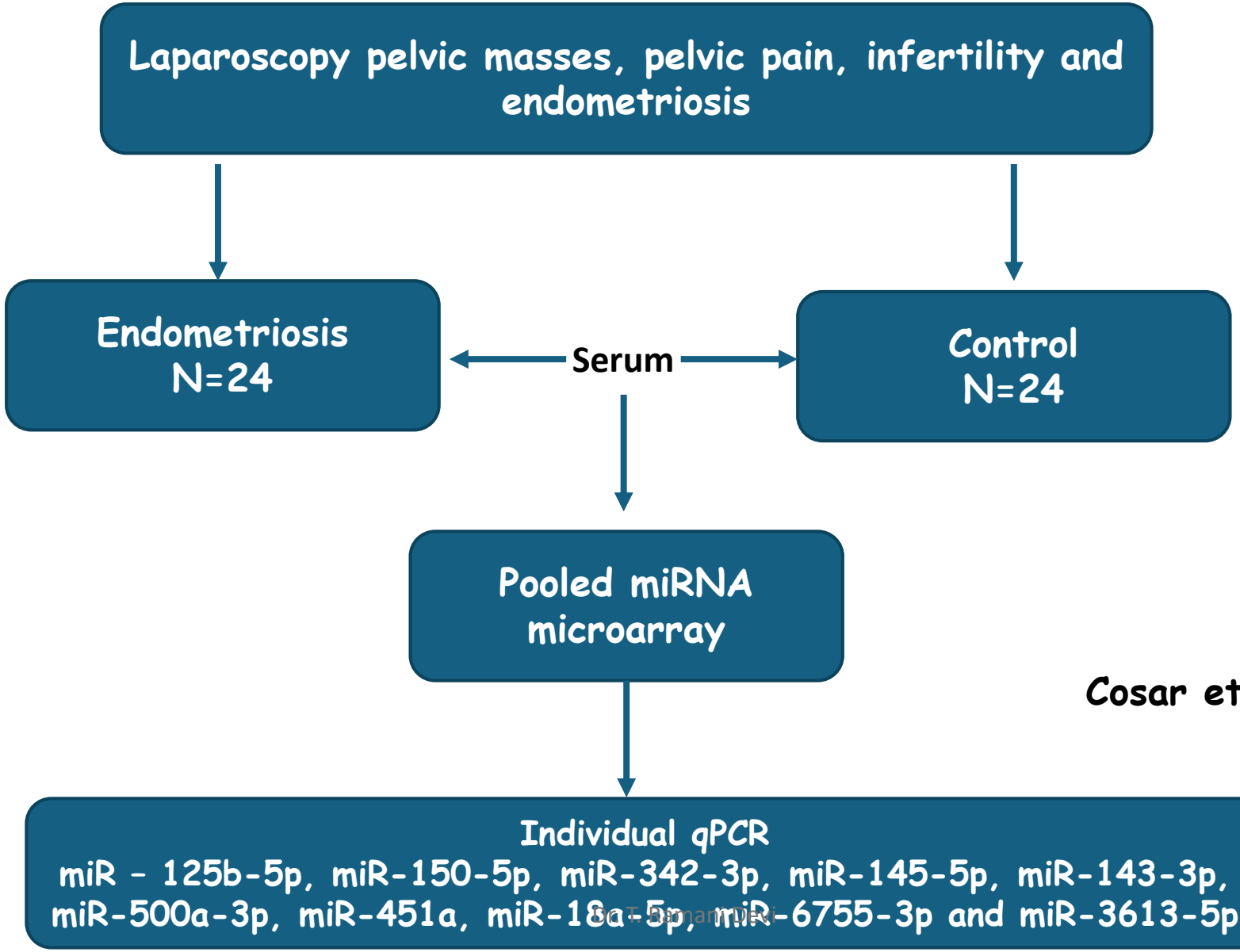


Dysregulated micro-RNAs in endometriosis

Sensitivity & specificity of dysregulated mi RNAs in endometriosis

Author, Reference	Dysregulated miRNA	Specificity	Sensitivity
Ohlsson et al., 2009 [127]	miR-200a, miR-200b, and miR-141	66,7%	84,4%
Jia et al., 2013 [134]	miR-22	90%	90%
	miR-17-5p	80%	60%
	miR-20a	90%	60%
	miR-145	96%	70%
Wang et al., 2013 [129]	miR-122	76%	80%
	miR-199a	76%	78,3%
	miR-141-5p	96%	71,7%
Suryavanshi et al., 2013 [135]	miR-195, miR-16, miR-191	60%	88%
Rekker et al., 2015 [131]	miR-200a-3p	70.8%	71.9%
	miR-200b-3p	90.6%	70.8%
	miR-141-3p	70.8%	71.9%
Cosar et al., 2016 [136]	miR-125b	96%	100%
Nisenblat et al., 2019 [132]	miR-155, miR574-3p and miR139-3p	51%	83%
Vanhie et al., 2019 [133]	hsa-miR-125b-5p, hsa-miR-28-5p and hsa-miR-29a-3p	37%	78%

mi-RNA as potential biomarkers?



Cosar et al., Fertil Steril 2016

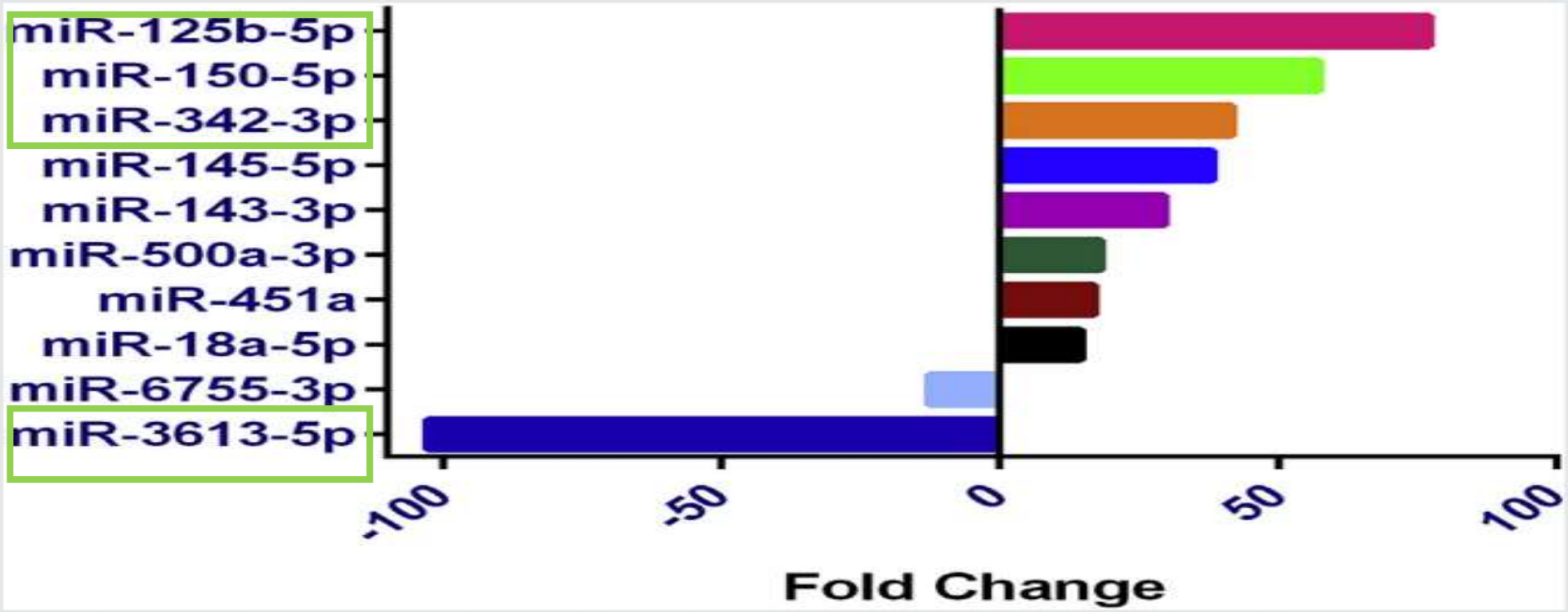
Fertility and Sterility asrm

ORIGINAL ARTICLE | VOLUME 106, ISSUE 2, P402-409, AUGUST 01, 2016

Serum microRNAs as diagnostic markers of endometriosis: a comprehensive array-based analysis

Emine Cosar, M.D. • Ramanaiah Mamillapalli, Ph.D. • Gulcin Sahin Ersoy, M.D. • SihYun Cho, M.D. • Benjamin Seifer, B.S. • Hugh S. Taylor, M.D.

Published: May 11, 2016 • DOI: https://doi.org/10.1016/j.fertnstert.2016.04.013 • Check for updates



Differentially expressed microRNAs in the microarray analysis.

mi-RNA as potential biomarkers?

Area under the receiver operating characteristic curve (AUC), 95% confidence interval (CI), and *P* values of the differentially expressed microRNAs.

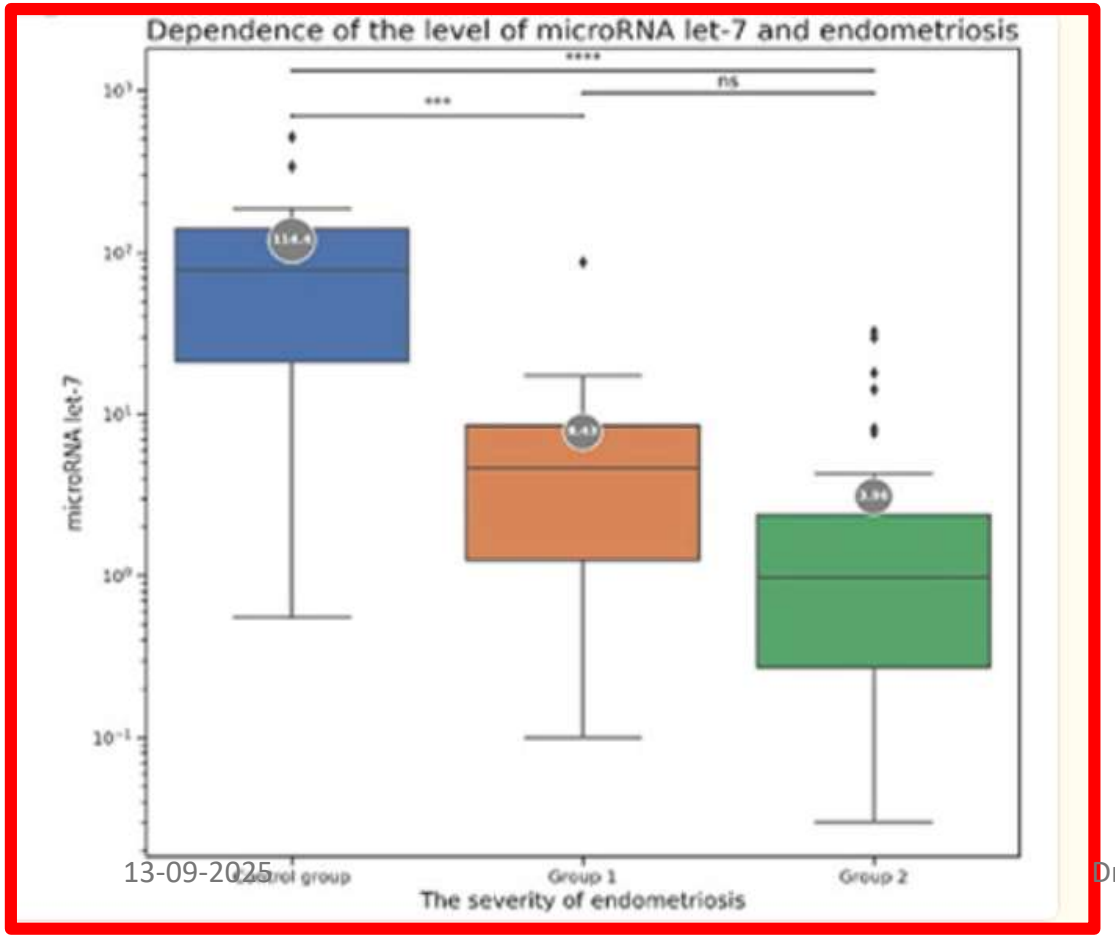
microRNA	AUC	95% CI	*P* value
miR-125b-5p	0.974	0.000–1.000	<.001
miR-150-5p	0.808	0.680–0.936	<.001
miR-342-3p	0.760	0.608–0.912	.002
miR-143-3p	0.926	0.000–1.000	<.001
miR-500a-3p	0.901	0.803–0.998	<.001
miR-451a	0.835	0.707–0.963	<.001
miR-18a-5p	0.797	0.653–0.940	.001
miR-6755-3p	0.718	0.577–0.860	.008
miR-3613-5p	0.862	0.740–0.985	<.001

Cosar. Serum microRNAs as endometriosis biomarkers. Fertil Steril 2016.

Turk J Obstet Gynecol. 2021 Dec; 18(4): 291–297.
Published online 2021 Dec 24. doi: 10.4274/tjod.galenos.2021.07277
PMCID: PMC8711670
PMID: 34955008

MicroRNA let-7: A promising non-invasive biomarker for diagnosing and treating external genital endometriosis

Darya A. Pokrovenko,[1] Volodymyr Vozniuk,[2] and Mykhailo V. Medvediev[3,*]



Conclusion:

MicroRNA let-7 had the best parameters (sensitivity, specificity, and predictive value of positive and negative results) among the biomarkers studied.

These biomarkers may be used for early and sometimes preclinical diagnosis of endometriosis.

Al Ameen J Med Sci 2022;15(4):330-339 • US National Library of Medicine enlisted journal • ISSN 0974-1143

ORIGINAL ARTICLE

CODEN: AAJMBG

Role of miRNA in non-invasive diagnosis of Endometriosis – A pilot study

T. Ramani Devi[1], Balamuthu Kadalmani[2] and C. Anchana Devi[3*]

[1]Research scholar, Department of Animal Science, Consultant Obstetrician & Gynaecologist, Ramakrishna Medical Centre LLP, 20-21, Vivekananda Nagar, Woraiyur, Trichy-620003, Tamil Nadu, India, [2]Department of Animal Science, Reproductive Endocrinology Lab, Bharathidasan University, Palkalaiperur, Tiruchirappalli-620024, Tamil Nadu, India and [3]Department of Biotechnology, Women's Christian College (Affiliated to University of Madras), College Road, Opp Standard Chartered Bank, Chennai-600006, Tamil Nadu, India

Received: 25th April 2022; ***Accepted:*** 23rd July 2022; ***Published:*** 01st October 2022

Abstract: *Background:* The Background of the study is to find out whether a panel of miRNAs from the serum of patients could be used as a biomarker for non-invasive diagnosis of endometriosis. *Objective:* A panel of miRNA 125b, miRNA150-5p, miRNA 342-3p, miRNA 3613-5p and Let-7b were studied in both control group who are found to be non-endometriotic patients and in the study group proven to be endometriotic patients. The objective is to find out whether these miRNAs could be used as a biomarker for diagnosis of endometriosis. *Methodology:* This study was done at Ramakrishna Medical Centre LLP. Patients undergoing laparoscopy for infertility and pain having endometriosis served as the study group and non-endometriotic patients served as the control group. Being a pilot study, we have included only 25 patients in each group. Based on the previous studies, a panel of 5 miRNAs were analysed from the serum of the women. Fasting sample was collected, serum was separated and cryo- preserved. q-RT-PCR analysis of the miRNA was done as per the protocols by a scientific team and results were analysed. Statistical analysis was done by generation of AUC. *Results:* miRNA 125b, miRNA 150-5p, miRNA 342-3p, miRNA 3613-5p and Let-7b were studied in both control and study group. There was up regulation of miRNA125b and miRNA342-3p and down regulation of Let-7b with the significant p value of <0.001. *Conclusion:* This pilot study shows that the panel of these miRNAs in peripheral circulating serum could be used as a non-invasive diagnostic marker for endometriosis.

Keywords: Endometriosis; Non-invasive diagnosis; Biomarkers; miRNA;

Combinatorial serum miRNA biomarkers as a non-invasive diagnostic tool for Endometriosis

Dr. T. Ramanidevi, MD, DGO.,
Ramakrishna Medical Centre LLP, Trichy, Tamil nadu

FIGO XXIV WORLD CONGRESS OF GYNECOLOGY AND OBSTETRICS

Aim

- To explore the performance of miRNA in the diagnosis of endometriosis.

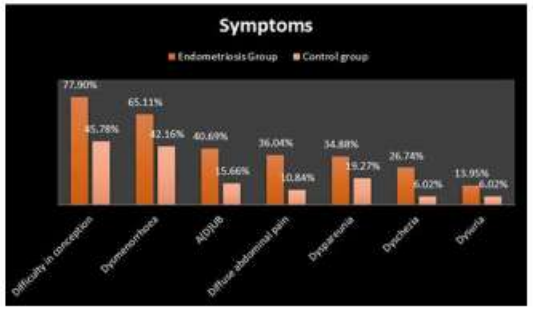
Material and Methods

- Prospective blinded study conducted at Ramakrishna Medical Centre LLP, Trichy, Tamil Nadu, between July 2021 – July 2022.
- Sample size: Study (Endometriosis) group (n=86), Control (Non-endometriosis) group (n=83).
- miRNA's analysed: miRNA 125b, 150-5p, 342-3p, 3613-5p & Let7b.

Methodology

- Fasting blood Sample during the proliferative phase was taken and serum separated and kept in liquid nitrogen - 196° C.
- miRNA extraction & analysis done by qRT- PCR.
- Machine learning with Random Forest (RF) approach along with 10-fold cross – Validation data analyzed.
- ROC, AUC were calculated.

Results

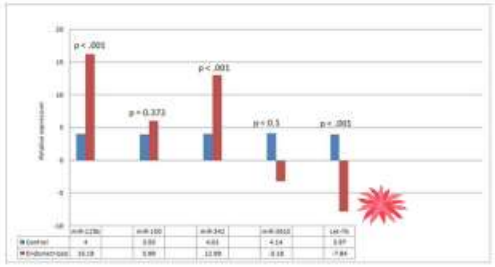


Symptoms	Endometriosis group (n=86) Mean ± SD (%)	Control group (n=83) Mean ± SD (%)	OR value	95% CI Value
Dysmenorrhoea	56 ± 0.65 (65.11%)	35 (42.16%)	1.54	0.92
Dyspareunia	30 ± 0.34 (34.88%)	16 (19.27%)	1.81	0.92
Dyschezia	23 ± 0.26 (26.74%)	5 (6.02%)	4.44	1.51
Dysuria	12 ± 0.13 (13.95%)	5 (6.02%)	2.32	0.78
DUBI	35 ± 0.40 (40.69%)	13 (15.66%)	2.598	1.28
Diffuse abdominal pain	31 ± 0.36 (36.04%)	9 (10.84%)	3.32	1.49
Difficulty in conception	67 ± 0.77 (77.90%)	38 (45.78%)	1.70	1.03

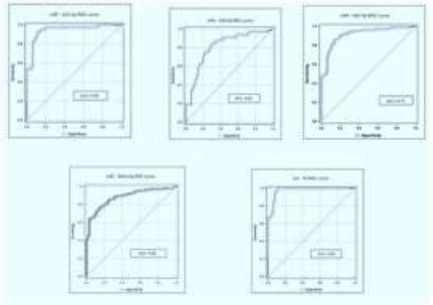
ROC analysis of each miRNA

miRNA	AUC (95% CI)	Cut-off value	Sensitivity (95% CI)	Specificity (95% CI)	Odds Ratio (95% CI)
miR-125b-5p	0.93 (0.89-0.97)	4.698*	0.87 (0.79-0.94)	0.83 (0.77-0.89)	23.69 (11.25-36.13)
miR-150-5p	0.66 (0.59-0.73)	2.261*	0.42 (0.36-0.48)	0.23 (0.17-0.28)	4.71 (2.44-6.97)
miR-342-3p	0.77 (0.72-0.81)	3.331*	0.74 (0.67-0.81)	0.74 (0.64-0.83)	16.83 (7.38-26.28)
miR-3613-5p	0.84 (0.76-0.91)	3.911*	0.87 (0.83-0.71)	0.33 (0.44-0.61)	12.09 (4.48-19.73)
Let-7b	0.90 (0.84-0.96)	5.079*	0.87 (0.81-0.93)	0.82 (0.71-0.92)	41.28 (14.38-68.19)

Dysregulation of miRNA



ROC / AUC Analysis



Various stages of endometriosis and dysregulation of mi RNAs

Discussion

- Let 7b and miRNA 3613-5p were downregulated; miRNA 125b and miRNA 342- 3p were upregulated.
- The expression of miRNAs did not differ as per phenotypes of endometriosis.
- Symptomatic subjects with no imaging based evidence of disease also tested positive test for early endometriosis. (6/86 study subjects)

Conclusion

- miRNAs are promising non-invasive biomarkers for endometriosis diagnosis, including early stages of the disease that cannot be detected by imaging or Laparoscopy,
- Serum miRNA assay can help in the diagnosis of endometriosis in patients who have symptoms suggestive of this condition, such as dysmenorrhea, dyschezia, and dyspareunia, but no image based evidence of the disease.
- Further research may help to improve diagnostic precision and assessment of response to therapy.

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- Bendifallah S, Suisse S, Puchar A, Delbos L, Poilblanc M, Descamps P, Golfier F, Jornea L, Bouteiller D, Touboul C, Dabi Y. Salivary MicroRNA Signature for Diagnosis of Endometriosis. Journ of Clinic Med 2022

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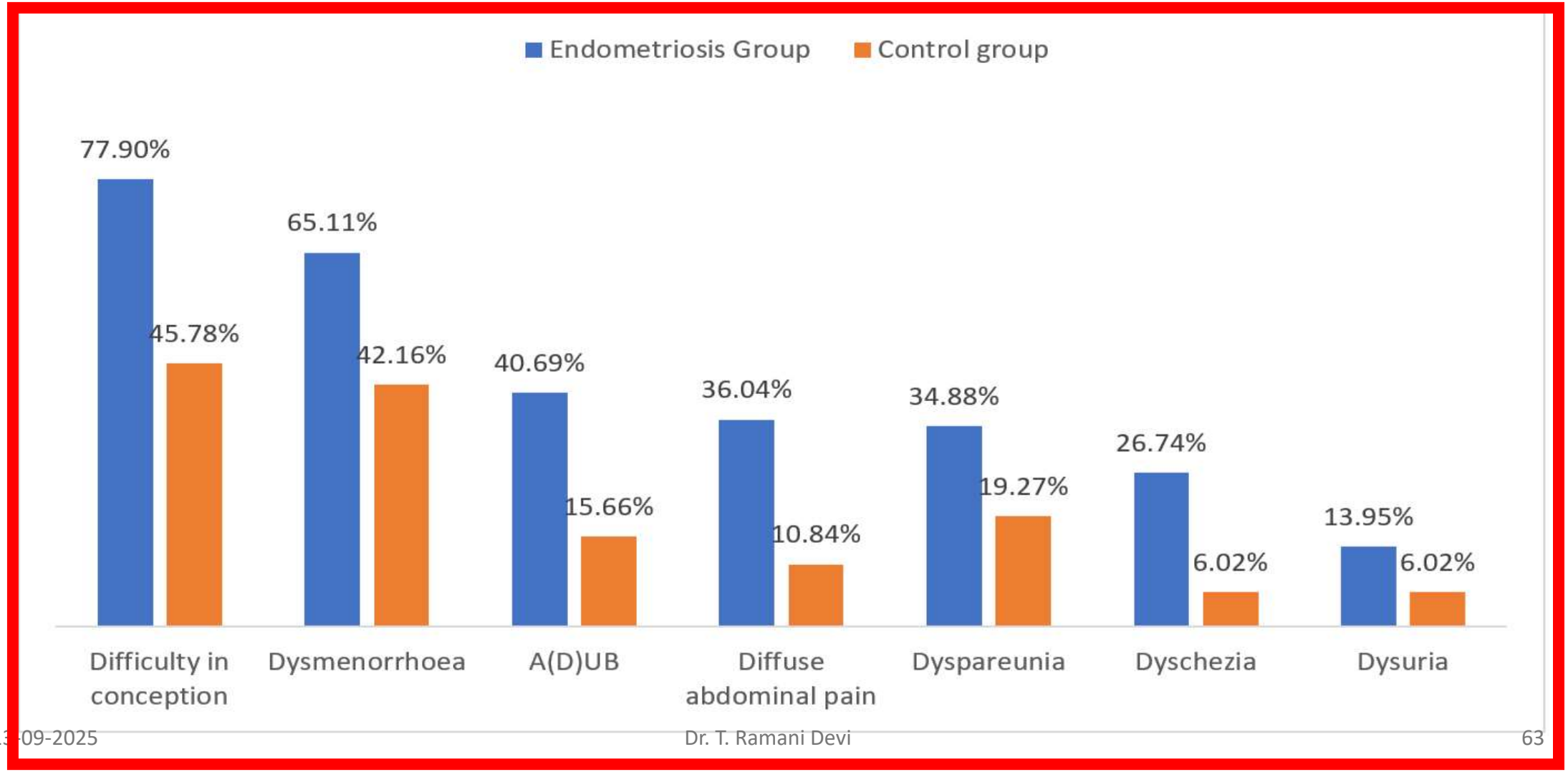
Demographic profile

Variables	Endometriosis group (n=86)	Control group (n=83)
Age	30.6± 5.57	29.9±4.85
BMI	25.9±4.59	27.3±4.25
rASRM Endometriosis stage		
Mild endometriosis (Stage I &II)	35/86(40.69%)	-
Moderate endometriosis (Stage III)	19/86(22.09%)	-
Severe endometriosis (Stage IV)	24/86 (27.90 %)	-
Suspicious of Endometriosis (Laparoscopy not done)	6/86 (6.97%)	

Menstrual history		
Regular cycles	60/86 (69.76%)	48/83(57.83%)
Irregular cycles	26/86 (30.23%)	35/83(42.16%)
Infertility		
Primary infertility	51/86(59.30%)	26/83(31.32%)
Secondary infertility	16/86(18.60%)	12/83(14.45%)

Symptoms


Endometriosis Group
Control group
77.90%
45.78%
65.11%
42.16%
40.69%
15.66%
36.04%
10.84%
34.88%
19.27%
26.74%
6.02%
13.95%
6.02%
Difficulty in conception
Dysmenorrhoea
A(D)UB
Diffuse abdominal pain
Dyspareunia
Dyschezia
Dysuria

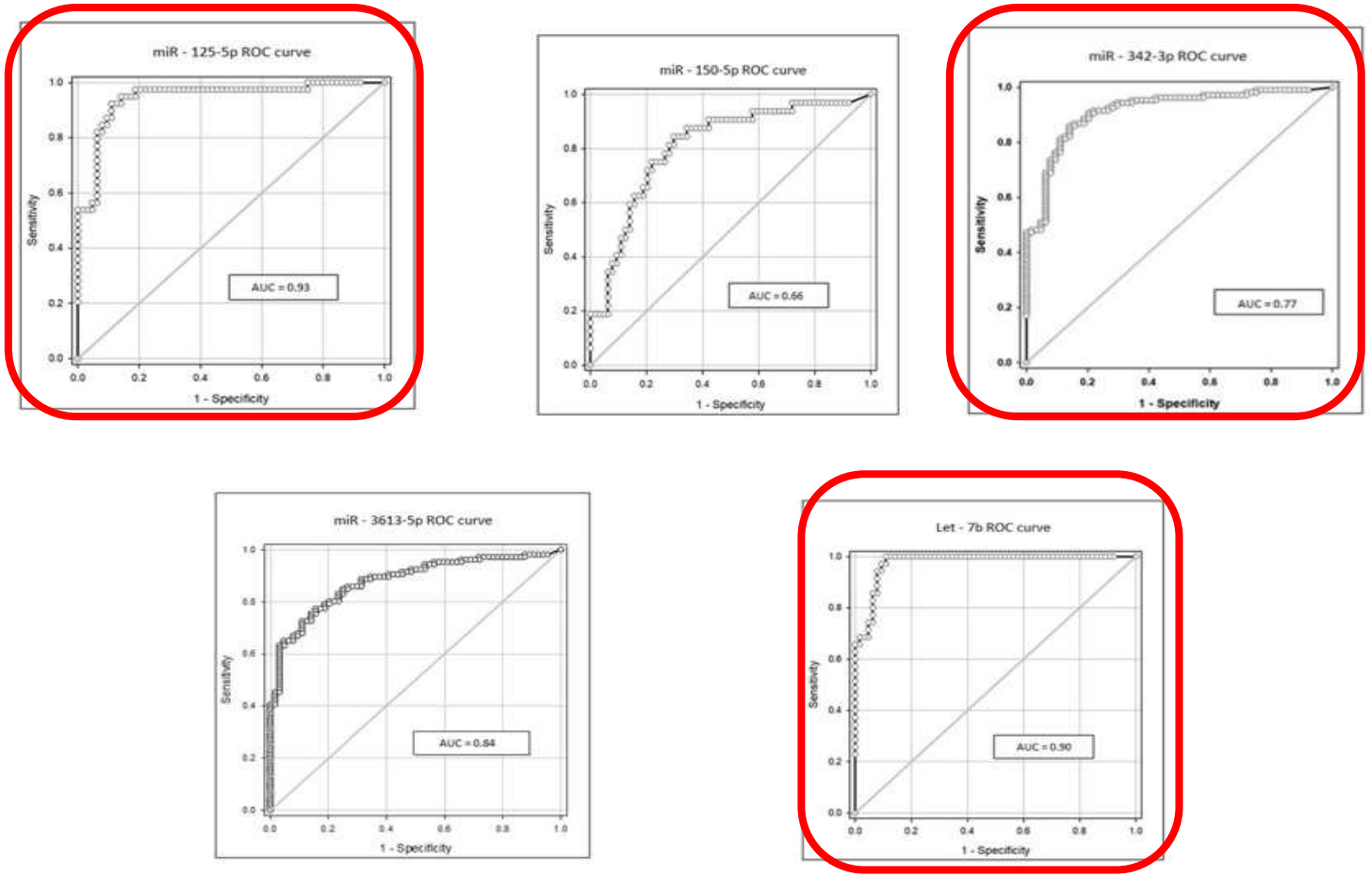
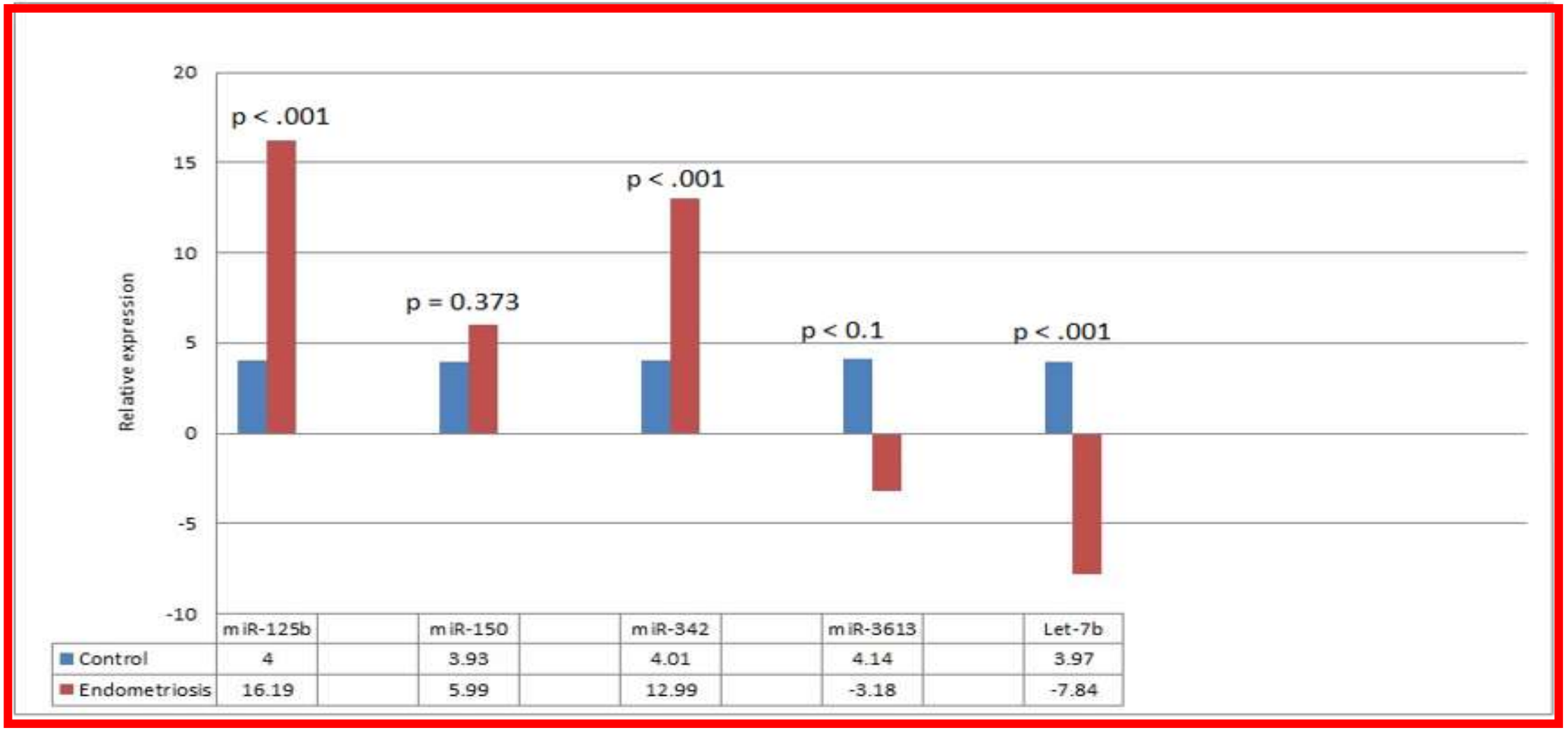


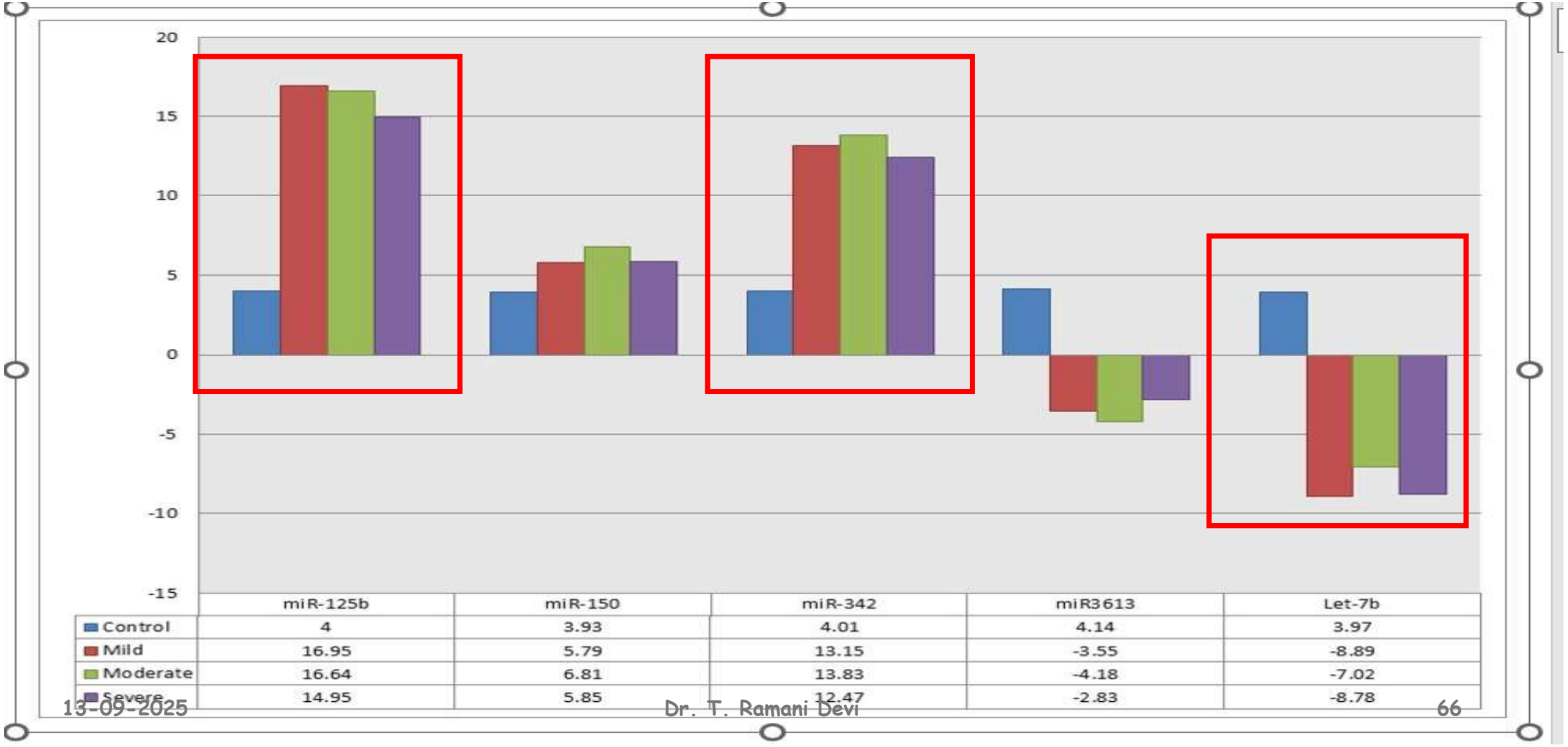
Fig 3: ROC Curves of miRNA 125b, miRNA 150-5p, miRNA 342 – 3p, miRNA 3613-5p and miRNA Let 7b

Statistical analysis



	miR-125b		miR-150		miR-342		miR-3613		Let-7b
Control	4		3.93		4.01		4.14		3.97
Endometriosis	16.19		5.99		12.99		-3.18		-7.84

Relationship between stages and mI-RNA



	miR-125b	miR-150	miR-342	miR3613	Let-7b
Control	4	3.93	4.01	4.14	3.97
Mild	16.95	5.79	13.15	-3.55	-8.89
Moderate	16.64	6.81	13.83	-4.18	-7.02
Severe	14.95	5.85	12.47	-2.83	-8.78

Results of our study

- **Results:** mi-RNA 125b, mi-RNA150-5p, mi-RNA 342-3p, mi-RNA 3613-5p and let-7b were studied in both control and study group. there was upregulation of mi-RNA125b and mi-RNA 342-3p and down regulation of let-7b with the significant p value of <0.001.

- **Conclusion:** This study shows that the panel of these mi-RNAs in serum could be used as a non-invasive diagnostic marker for endometriosis.

Summary of our study

- Based on the assay of these miRNAs and clinical findings, it is possible to diagnose endometriosis at an early stage.

- In our study, the expression of targeted miRNAs in endometriosis show no variation related to the stages of endometriosis.

- We need more samples to study the phenotype and miRNAs

ijlpr 2023; doi 10.22376/ijlpr.2023.13.4.L146-L157

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ISSN 2250-0480

Research Article

Serum miRNA Biomarker for Endometriosis

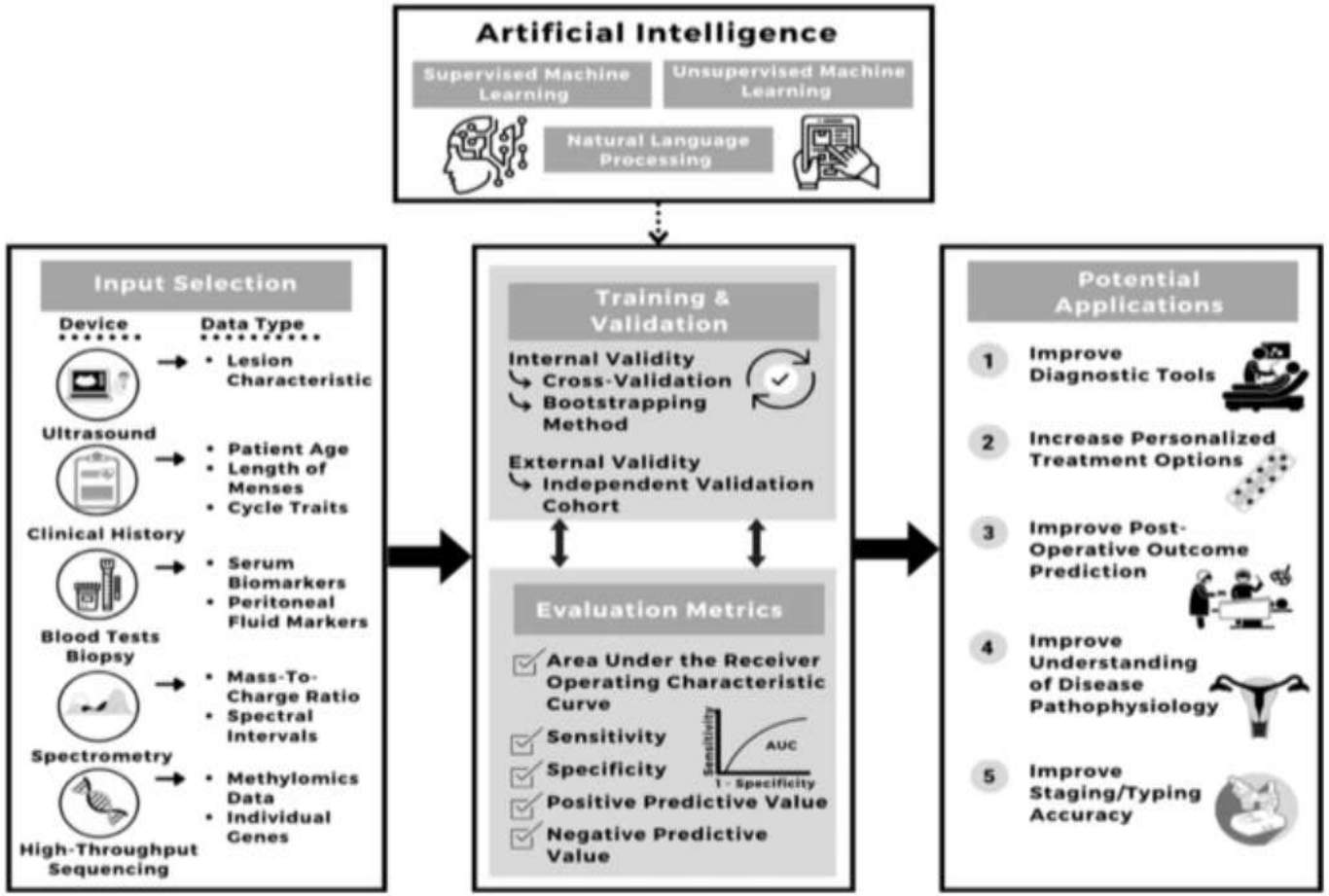
Comparative Study of Serum miRNA as A Biomarker for Non-Invasive Diagnosis in Suspected Versus Proven Cases of Endometriosis

Dr. T. Ramanidevi[1, 2], Dr. C. Anchana Devi[3], Dr. B. Kadalmani[1*] and Dr. Hari Balaji[4]

- **Aim:** The aim of the study is to find out whether a panel of miRNAs from the serum of patients and demographic profiling could be used as a biomarker for non-invasive diagnosis of endometriosis.
- **Objectives:** The objective is to find out whether these miRNAs could be used as a biomarker for non-invasive diagnosis of endometriosis.
- **Methodology:** A panel of miRNA 125b,miRNA 342-3p and Let-7b were studied in both control group (n 40) who are found to be non-endometriotic patients and in the study group (n 56) proven to be endometriotic patients. Along with the miRNAs study demographic profile in both groups were studied
- **Results:** Both groups exhibited dysregulation of mi RNAs.Hence this will reduce the time taken for diagnosis

.

Use of AI in endometriosis



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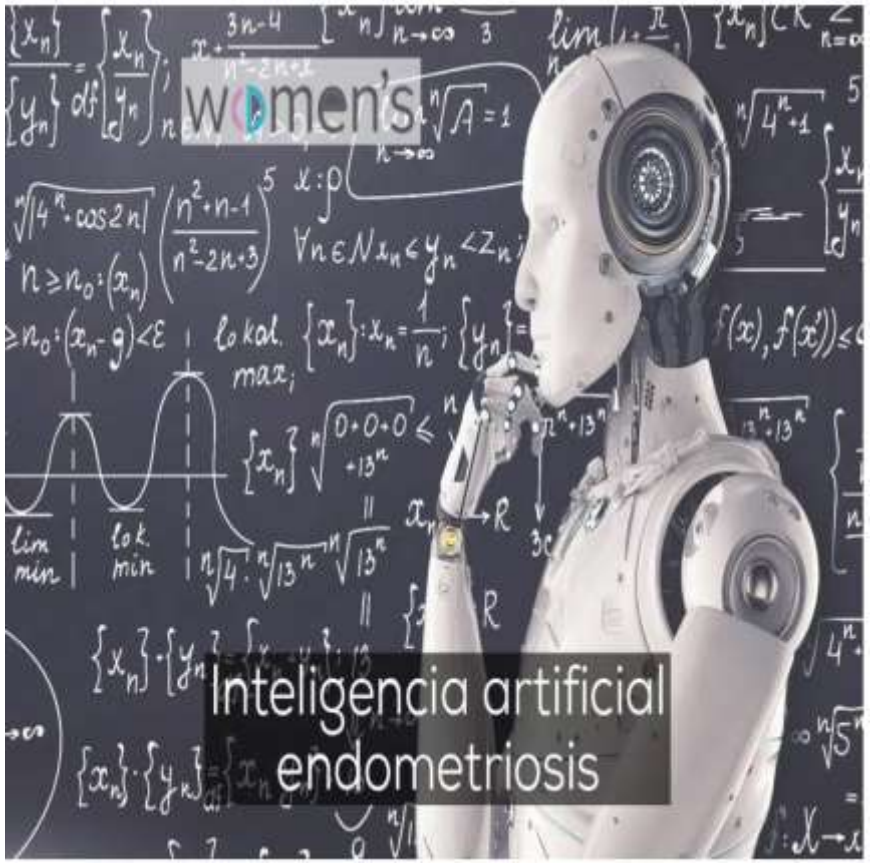

Yale Sophomore Helps to Develop Smart Tampon, Potential Way to Detect Endometriosis

DotEndo

Finally, the non-invasive test for endometriosis

Salivary based endo test based on mi RNAs

Conclusion

- Endometriosis is a common gynecological condition which leads to infertility and pain
- 10 -20 % of women of reproductive age suffer from endometriosis
- There is a diagnostic delay of about 8-9 years by which time disease would be advanced
- Clinical and non-invasive diagnosis are sufficient to start the treatment without HPE confirmation
- Imaging like USG and MRI may not help in diagnosis of early stages
- Currently we have biomarkers which can diagnose the disease much earlier
- Glyco-proteins, Growth factors, Inflammatory factors, angiogenic factors, oxidative stress markers and micro RNAs are some of them

Conclusion

- miR -125b, miR -342-3p were significantly upregulated in endometriosis subjects as compared to controls
- miRNA Let-7b were downregulated in endometriosis subjects as compared to controls. miRNA 3613- 5p was down regulated only with less significance.
- No change in levels of miR-150 & miR -3613 5p between endometriosis subjects and controls (contrary to published literature with western subjects)
- Based on the three results we concluded these 3 miRNAs- miR -125b, 342-3p and Let-7b as a promising biomarker for non invasive diagnosis of endometriosis irrespective of the stage of endometriosis

Conclusion

- This proves dysregulation of specific miRNAs are correlating well with the diagnosis of endometriosis.
- This can ultimately reduce the pain and prevent the progression of the lesion.
- Fertility is also not affected.
- Large databases of this miRNA study may play a key role to use this as a biomarker in future studies.
- Future studies can be extended towards the response of the disease for the hormonal treatment.
- We need larger prospective studies to evaluate the accuracy of diagnosis, treatment and outcome of the disease.

Ramakrishna Medical Centre

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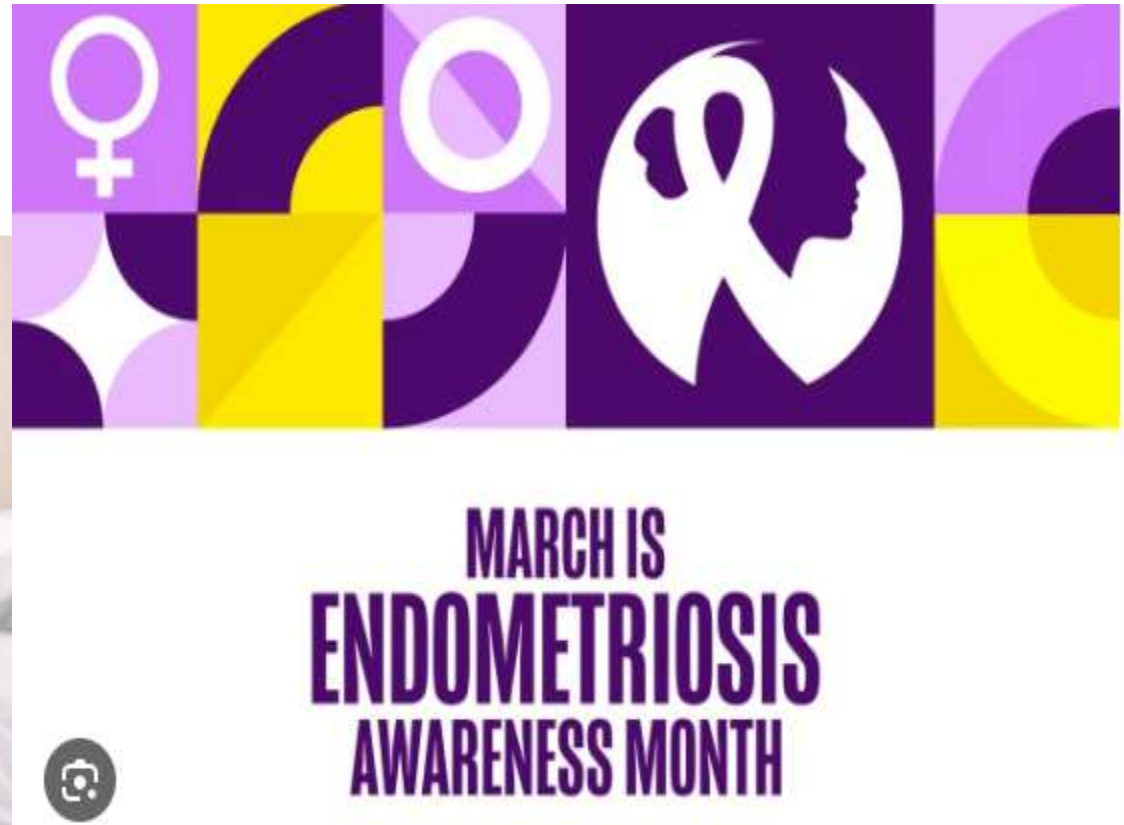
Endometriosis-Explained and "Let's Talk Endo"



Thanks To Our Team


thank
you


BHARATHIDASAN UNIVERSITY, TIRUCHIRAPPALLI
39-வது பட்டமளிப்பு விழா - 29.10.2024 - காலை 09.30
39th CONVOCATION - 29.10.2024 - 09.30