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Biomarkers for endometriosis

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The diagnostic challenge

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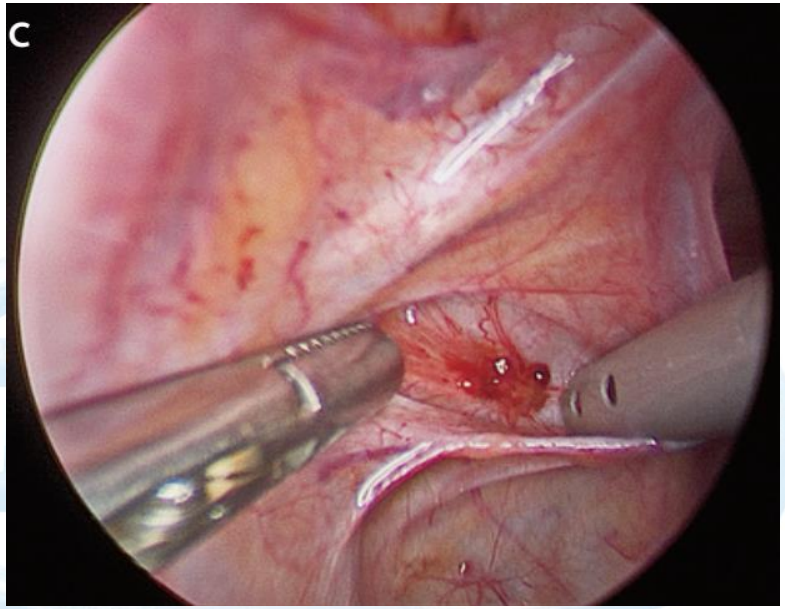
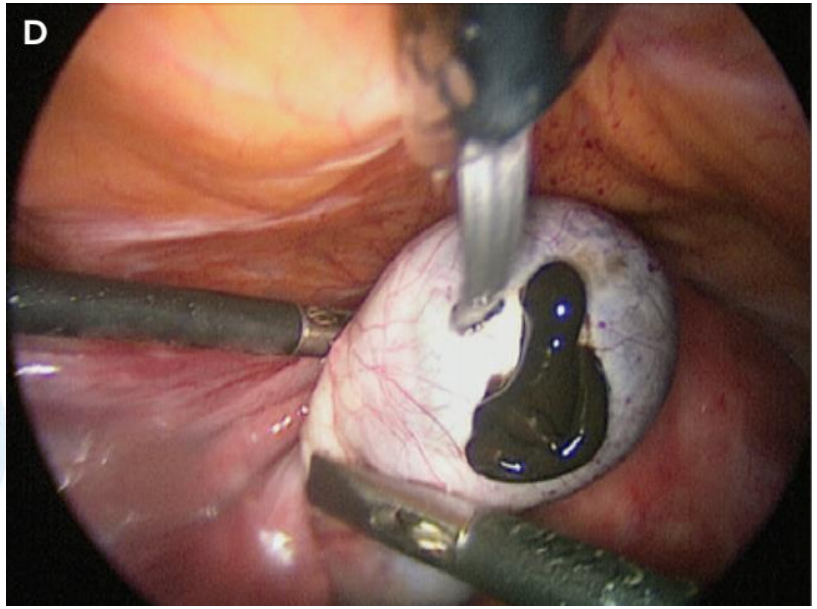
There is no such thing as ‘endometriosis’

Definition: presence of endometrial like tissue outside of the uterus

Peritoneal endometriosis



Endometrioma



Deep endometriosis

Zondervan et al. NEJM 2020

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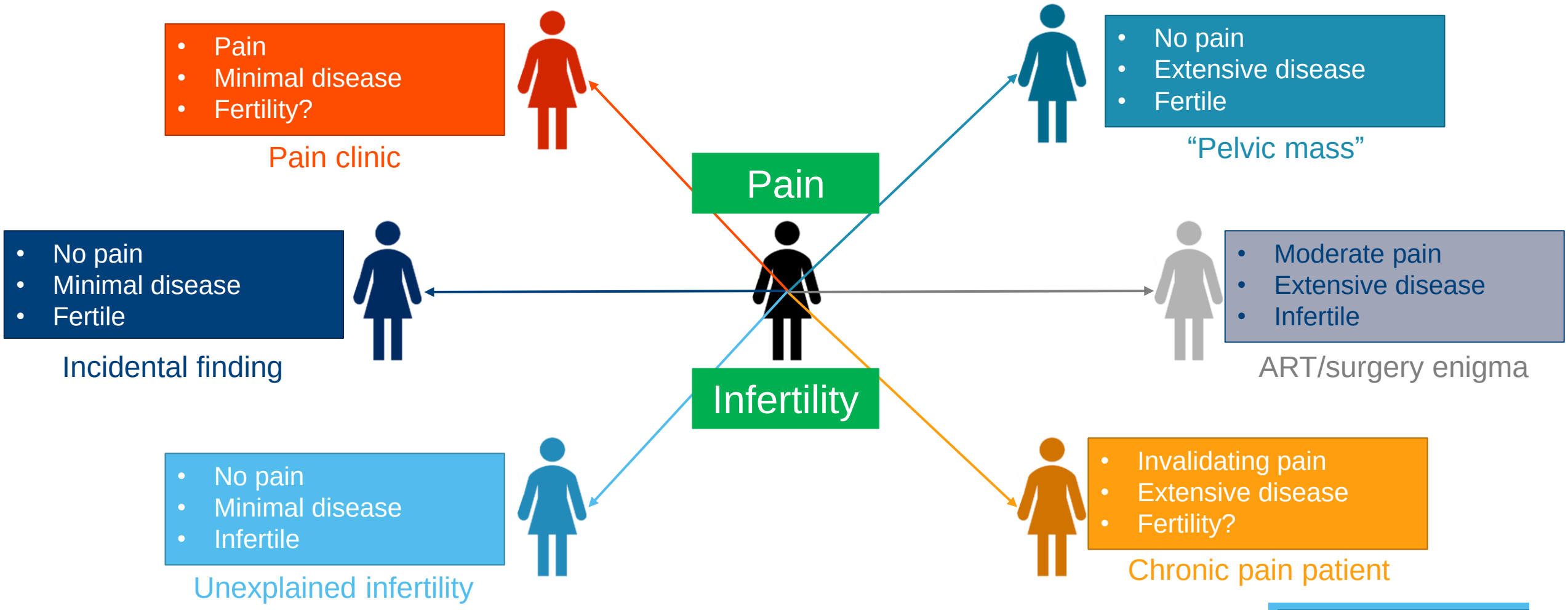
There is no such thing as ‘an endometriosis patient’



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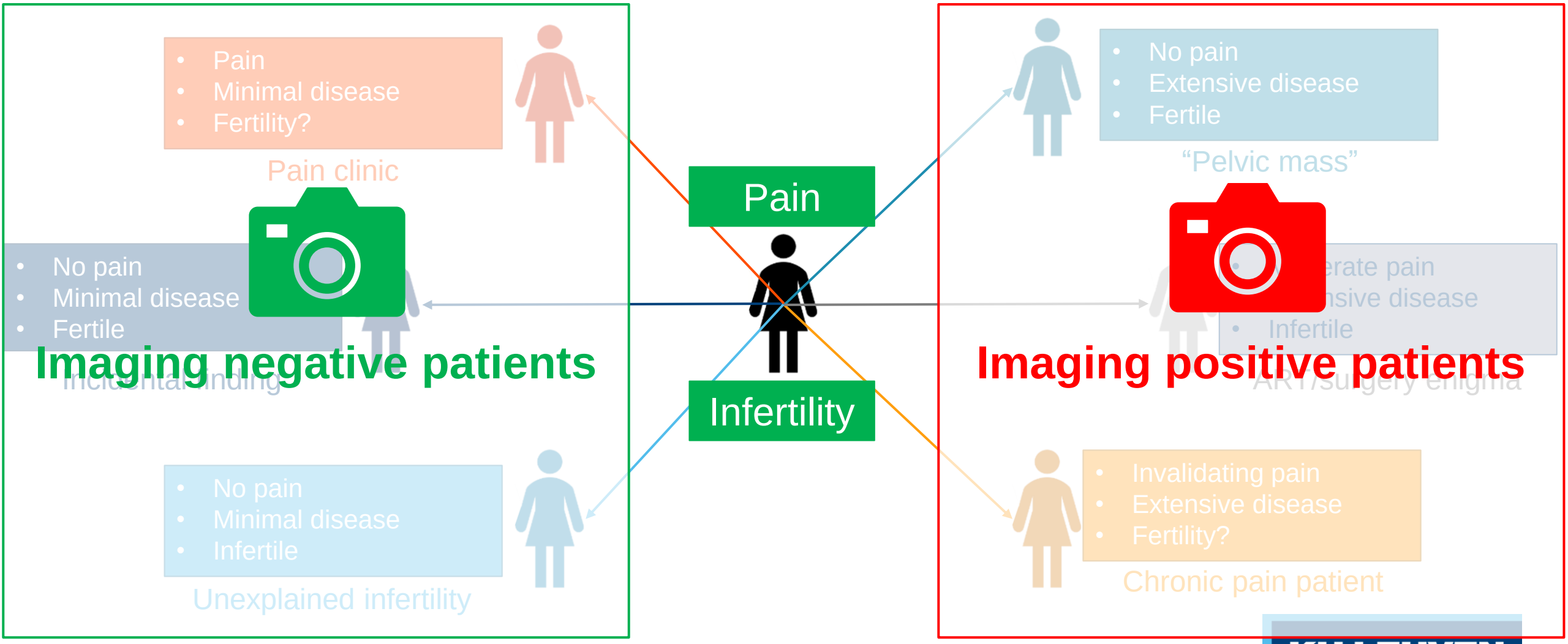
There is no such thing as ‘an endometriosis patient’



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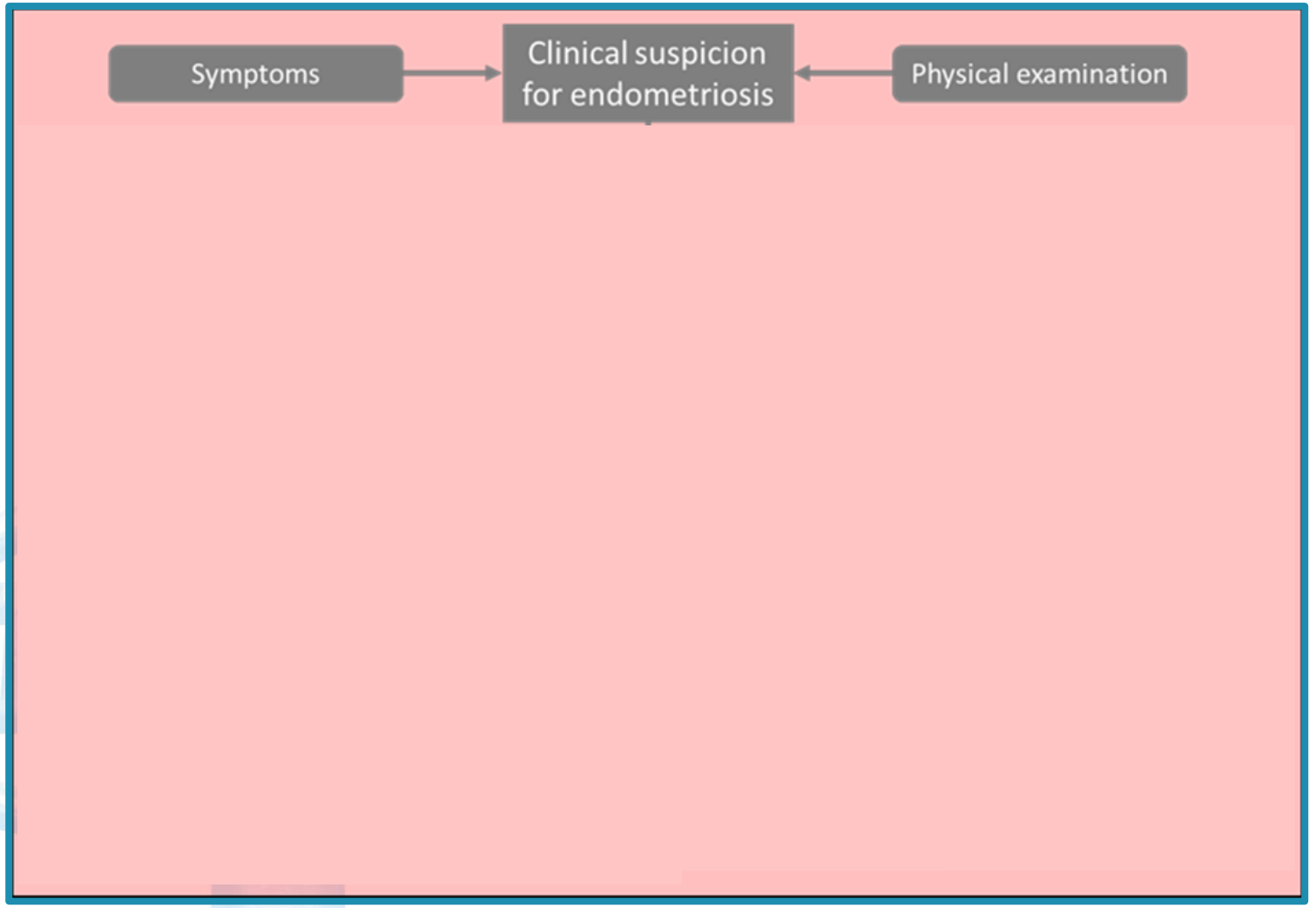
The role for non-invasive diagnosis



Red Flag Test

Pain or infertility
- ✓ Peritoneal + deep endometriosis
- ✓ False negative TVUS

Reduce Diagn Lap Test

Pain
- ✓ Peritoneal endometriosis
- ✓ Negative TVUS

Infertility + Pain
- ✓ Peritoneal endometriosis
- ✓ Negative TVUS

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Biomarkers for endometriosis: state of the art

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Definition

- WHO: *“any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease”*

- NIH: *“a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.”*

Different aspects of non-invasive diagnosis

Cochrane Library

Cochrane Database of Systematic Reviews

Imaging modalities for the non-invasive diagnosis of endometriosis (Review)

Nisenblat V, Bossuyt PMM, Farquhar C, Johnson N, Hull ML

Cochrane Library

Cochrane Database of Systematic Reviews

Endometrial biomarkers for the non-invasive diagnosis of endometriosis (Review)

Gupta D, Hull ML, Fraser I, Miller L, Bossuyt PMM, Johnson N, Nisenblat V

Cochrane Library

Cochrane Database of Systematic Reviews

Blood biomarkers for the non-invasive diagnosis of endometriosis (Review)

Nisenblat V, Bossuyt PMM, Shaikh R, Farquhar C, Jordan V, Scheffers CS, Mol BWJ, Johnson N, Hull ML

Cochrane Library

Cochrane Database of Systematic Reviews

Combination of the non-invasive tests for the diagnosis of endometriosis (Review)

Nisenblat V, Prentice L, Bossuyt PMM, Farquhar C, Hull ML, Johnson N

Cochrane Library

Cochrane Database of Systematic Reviews

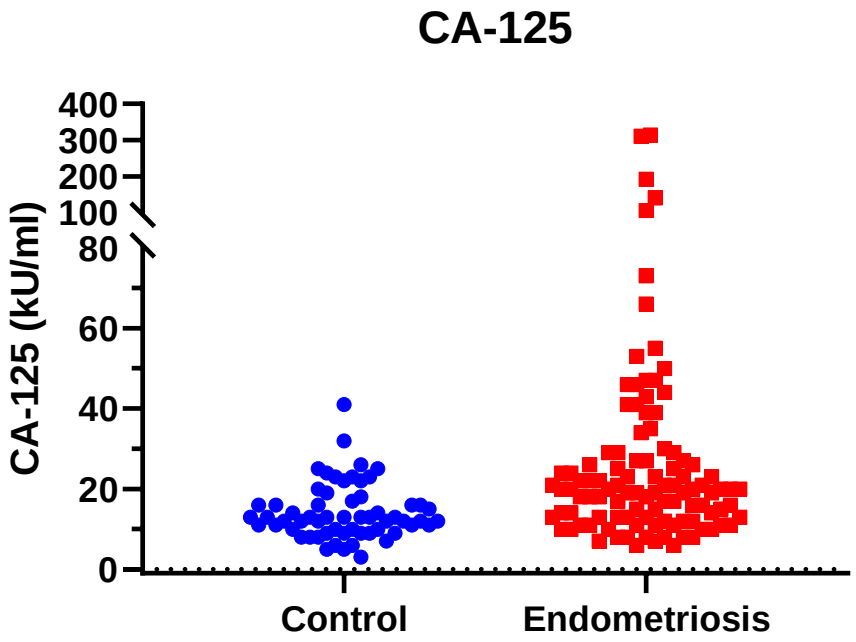
Urinary biomarkers for the non-invasive diagnosis of endometriosis (Review)

Liu E, Nisenblat V, Farquhar C, Fraser I, Bossuyt PMM, Johnson N, Hull ML

Many are called but few are chosen…

- CA-125
- Auto-antibodies
- Circulating miRNAs
- Endometrial nerve fibers
- Inflammatory markers
- ….

Biomarker ≠ diagnostic test



Biomarkers for endometriosis: circulating miRNA

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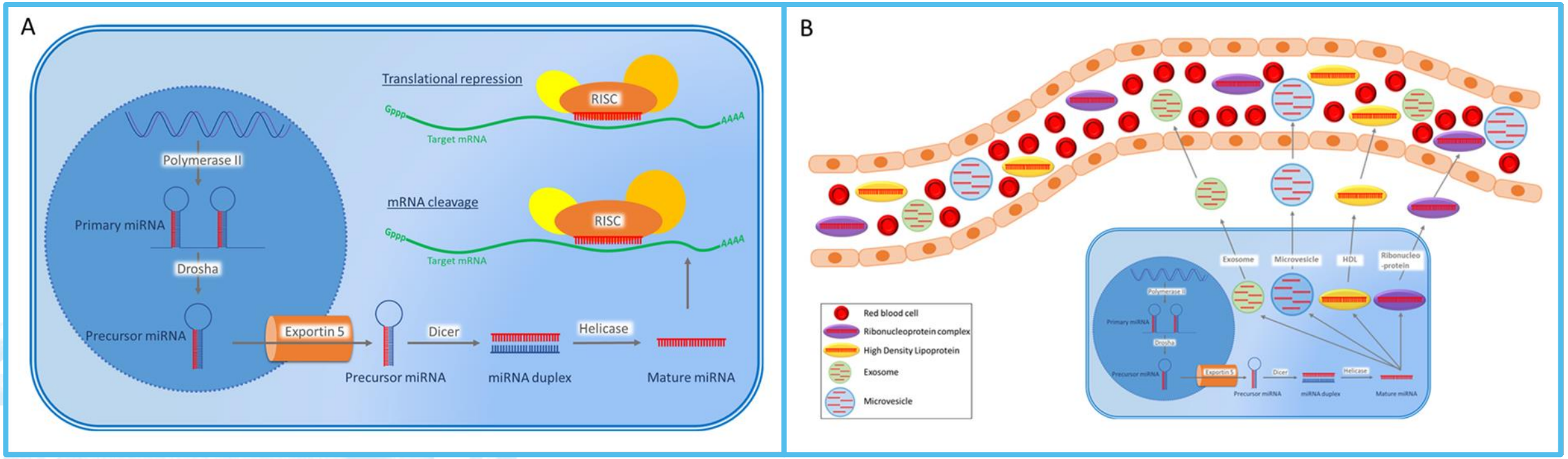
Why miRNAs?

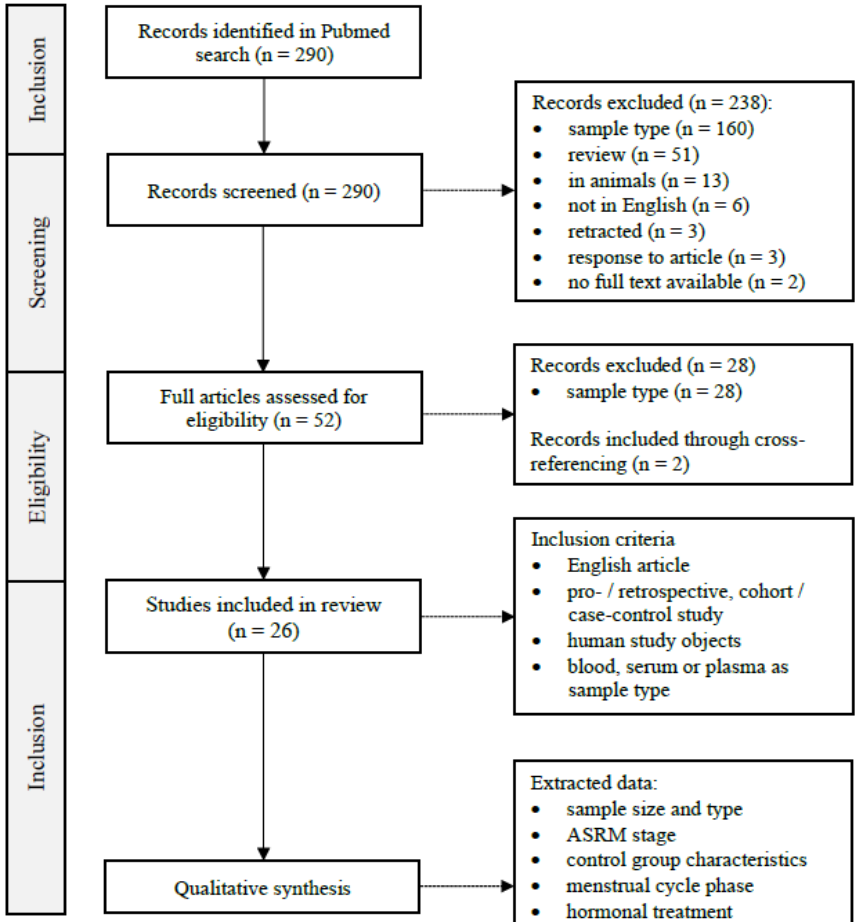


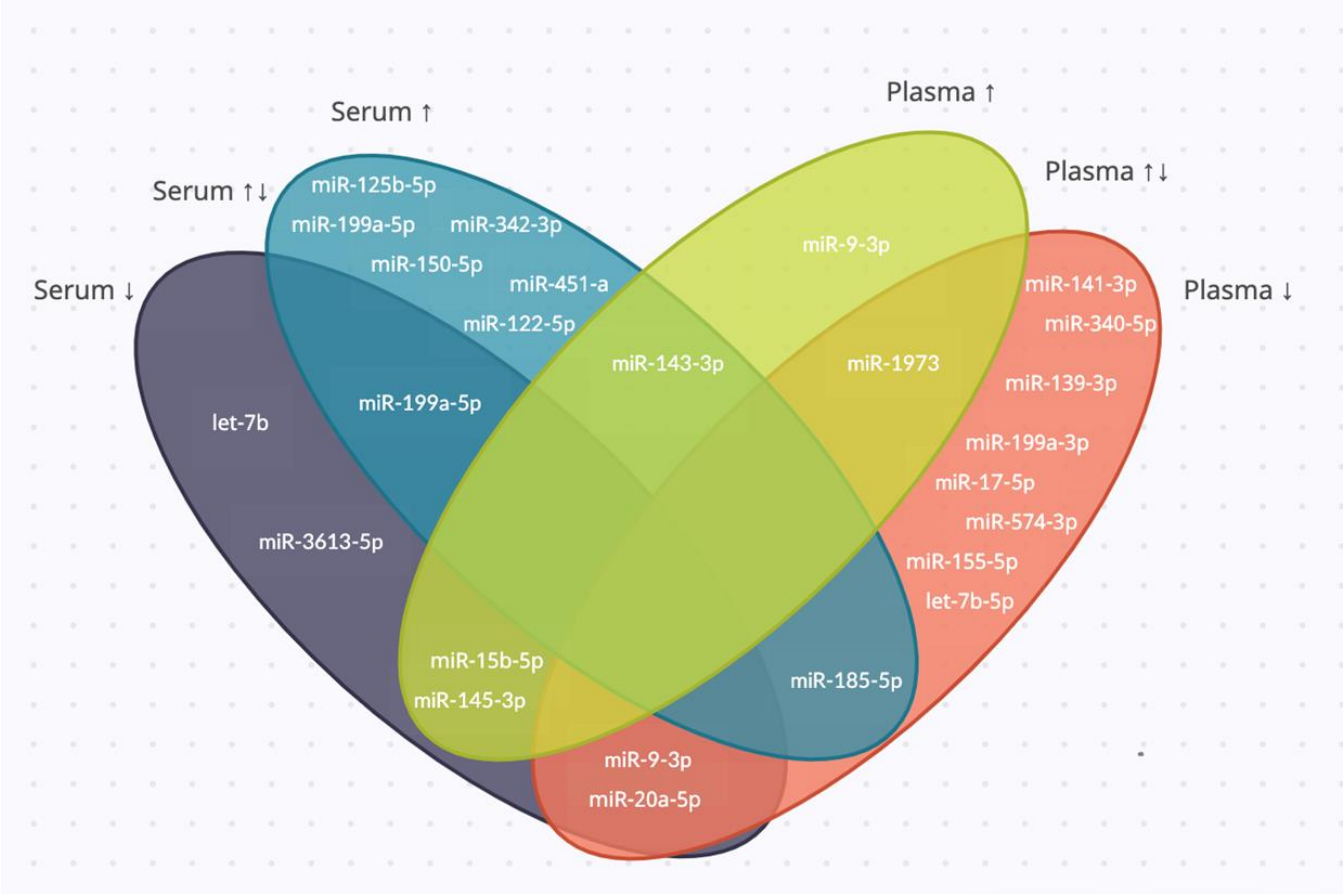
Small non-coding RNA

Stable in circulation

Limited overlap & conflicting results

Figure 1. PRISMA flowchart of literature search and study selection





Vanhie et al, under review

Nisenblat et al. JCEM 2019

- ✓ Plasma
- ✓ RT-qPCR (multiplex & singleplex)
- ✓ Explorative phase (n=16)
  - Healthy women (n=8) & endometriosis patients (n=8)
- ✓ Discovery phase (n=36)
  - Endometriosis vs asymptomatic women
  - Endometriosis vs surgically proven absence of endometriosis
- ✓ Selection phase (n=78)
  - Symptomatic women
- ✓ Validation phase (n=119)
  - Symptomatic women

Plasma miRNAs Display Limited Potential as Diagnostic Tools for Endometriosis

Victoria Nisenblat,[1,2] David J. Sharkey,[1,2] Zhao Wang,[1,2] Susan F. Evans,[3] Martin Healey,[4] E. Maria C. Ohlsson Teague,[1,2] Cristin G. Print,[5,6] Sarah A. Robertson,[1,2] and M. Louise Hull[1,2,7]

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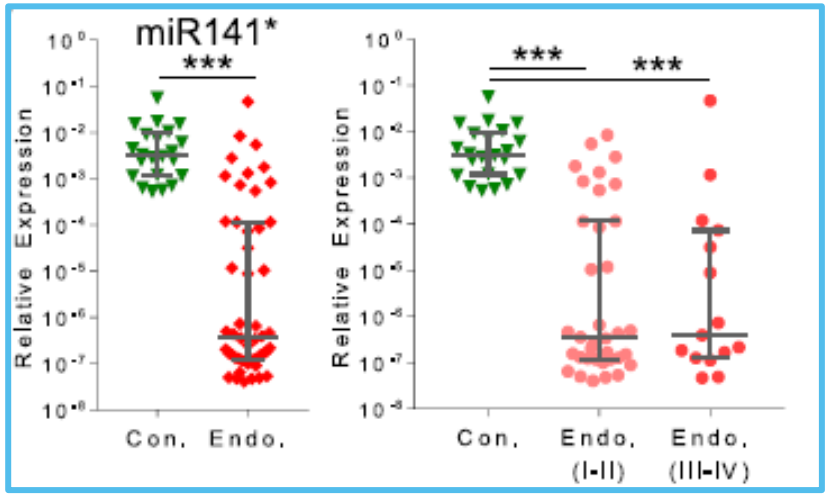
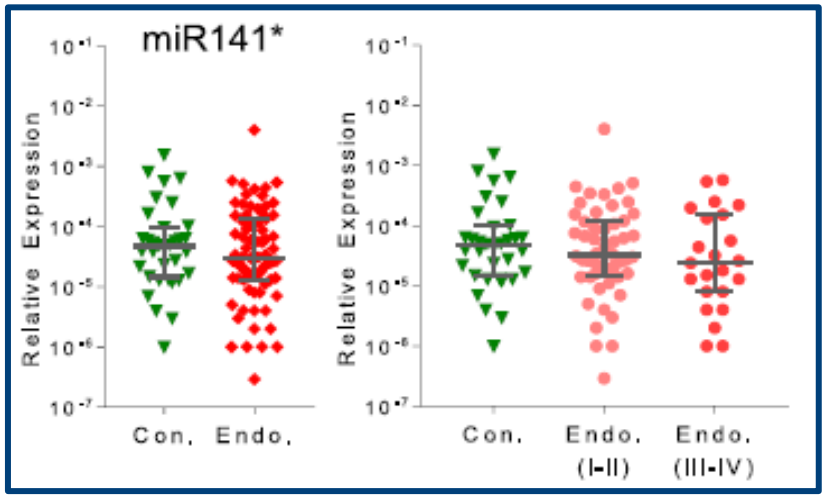
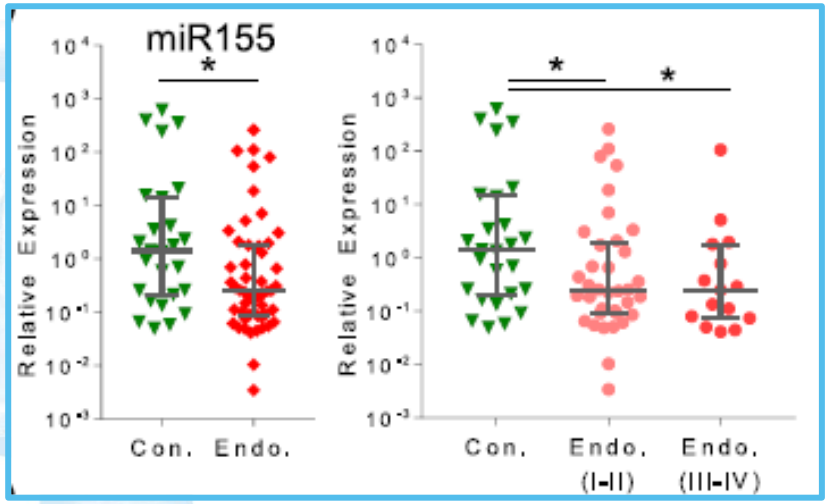
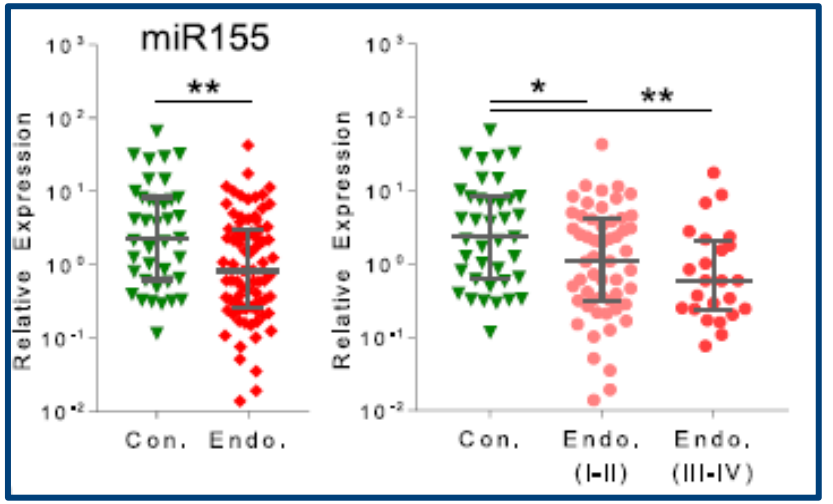
Nisenblat et al. JCEM 2019









Vanhie et al. Human Reproduction 2019

- ✓ NGS and RT-qPCR
- ✓ Plasma
- ✓ Discovery phase (n=120)
  - Symptomatic women
  - Laparoscopiclly proven
- ✓ Validation phase (n=90)
  - Symptomatic women
  - Laparoscopiclly proven
- ✓ Predominantly ultrasound negative + infertility & pain

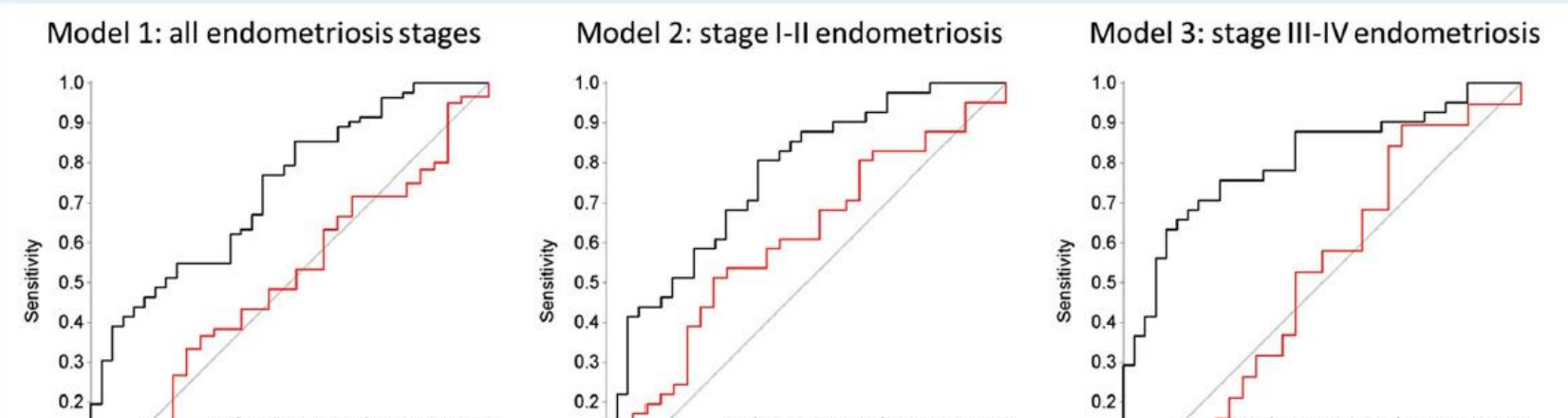
Human Reproduction, Vol.34, No.9, pp. 1650–1660, 2019
Advance Access Publication on August 14, 2019 doi:10.1093/humrep/dez116

human reproduction ORIGINAL ARTICLE *Gynaecology*

Plasma miRNAs as biomarkers for endometriosis

A. Vanhie[1,2,*], D. O[1], D. Peterse[1], A. Beckers[3], A. Cuéllar[3], A. Fassbender[1], C. Meuleman[1,2], P. Mestdagh[3,4,5], and T. D'Hooghe[1]

Vanhie et al. Human Reproduction 2019



	Control	Endometriosis	
Test negative	11/29 (38%)	23/60 (38%)	NPV: 32%
Test positive	18/29 (62%)	37/60 (62%)	PPV: 67%
	Sens:62%	Spec:38%	Accuracy: 54%

	Control	Endometriosis	
Test negative	11/30 (37%)	9/41 (22%)	NPV: 55%
Test positive	19/30 (63%)	32/41 (78%)	PPV: 63%
	Sens:78%	Spec:37%	Accuracy: 61%

	Control	Endometriosis	
Test negative	13/30 (43%)	8/19 (42%)	NPV: 62%
Test positive	17/30 (57%)	11/19 (58%)	PPV: 39%
	Sens:58%	Spec:43%	Accuracy: 49%

Figure 2 Validation of the diagnostic models in an independent patient cohort. Black curve = Discovery cohort; Red curve = validation cohort. Data in the contingency tables are from the validation cohort.

Model 1
- Let-7d-5p
- miR-21-5p
- miR-28-5p

Model 2
- miR-125b-5p
- miR-28-5p
- miR-29a-3p

Model 3
- miR-21-5p
- miR-28-5p
- miR-30a-5p

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Moustafa et al. AJOG 2020

- ✓ Serum
- ✓ RT-qPCR
- ✓ Discovery phase
  - Cosar et al 2016 Fertil Steril
- ✓ Model building phase (n=100)
  - Indication: suspected benign conditions
- ✓ Validation phase (n=48)
  - Retrospective validation in existing dataset (Cosar et al 2016 Fertil Steril)

Moustafa et al. AJOG 2020

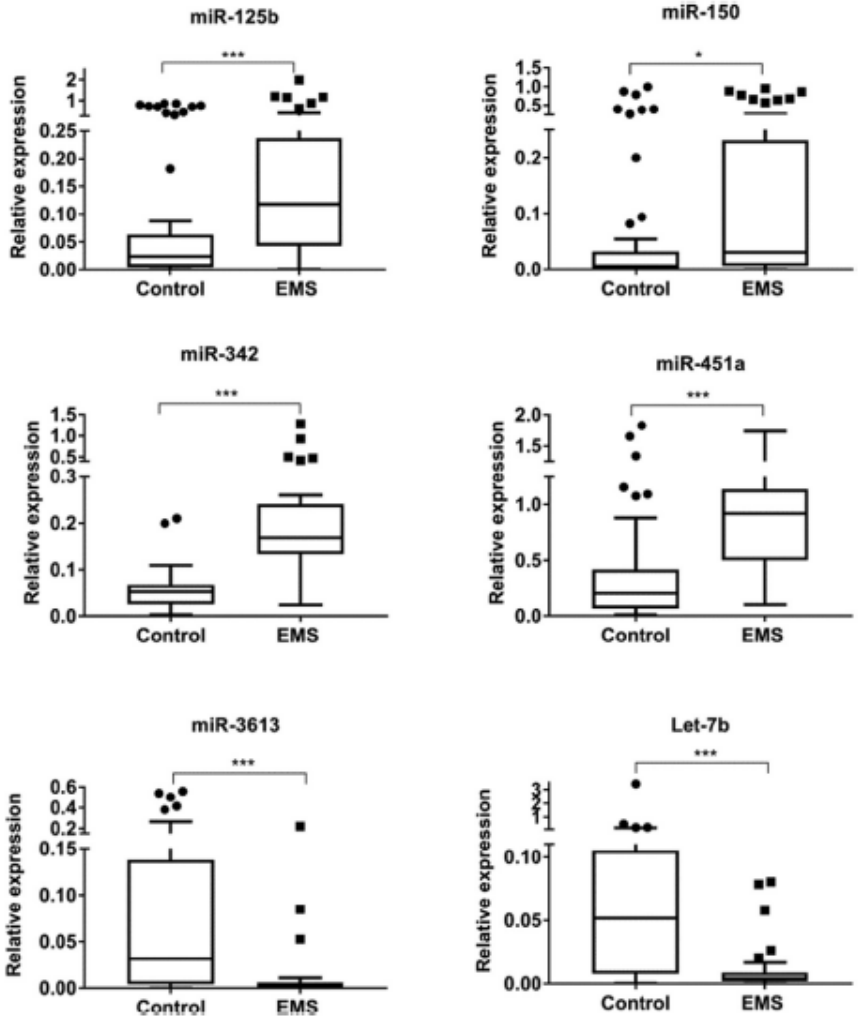
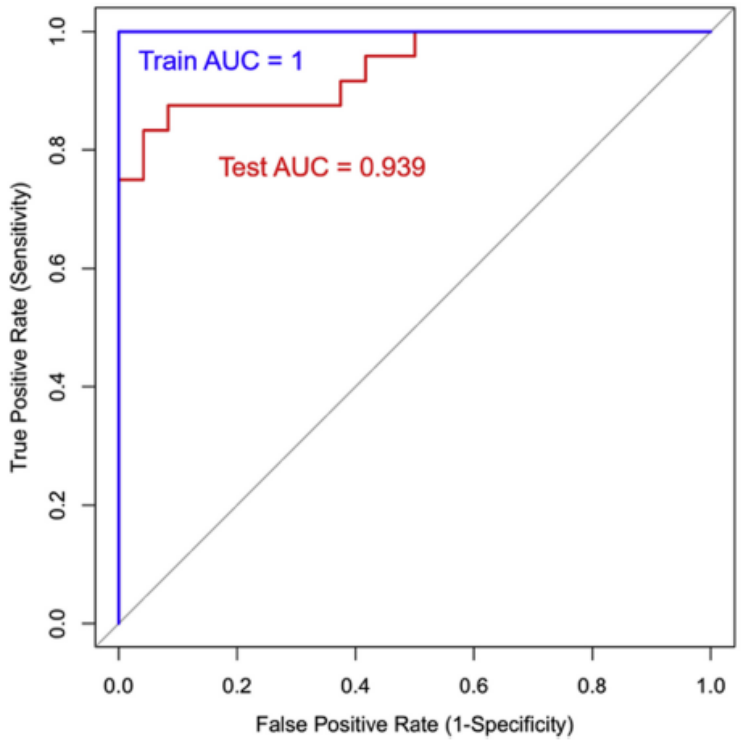


FIGURE 4
Performance of classifier algorithm in training and independent data set



Receiver-operating characteristic analysis of the random forest model using 6 miRNA biomarkers (miR-125b-5p, miR-150-5p, miR-342-3p, miR-451a, miR-3613-5p, let-7b). The model was derived in the current (n = 100) data set (Train) and tested in an independent cohort (n = 48) data set (Test).

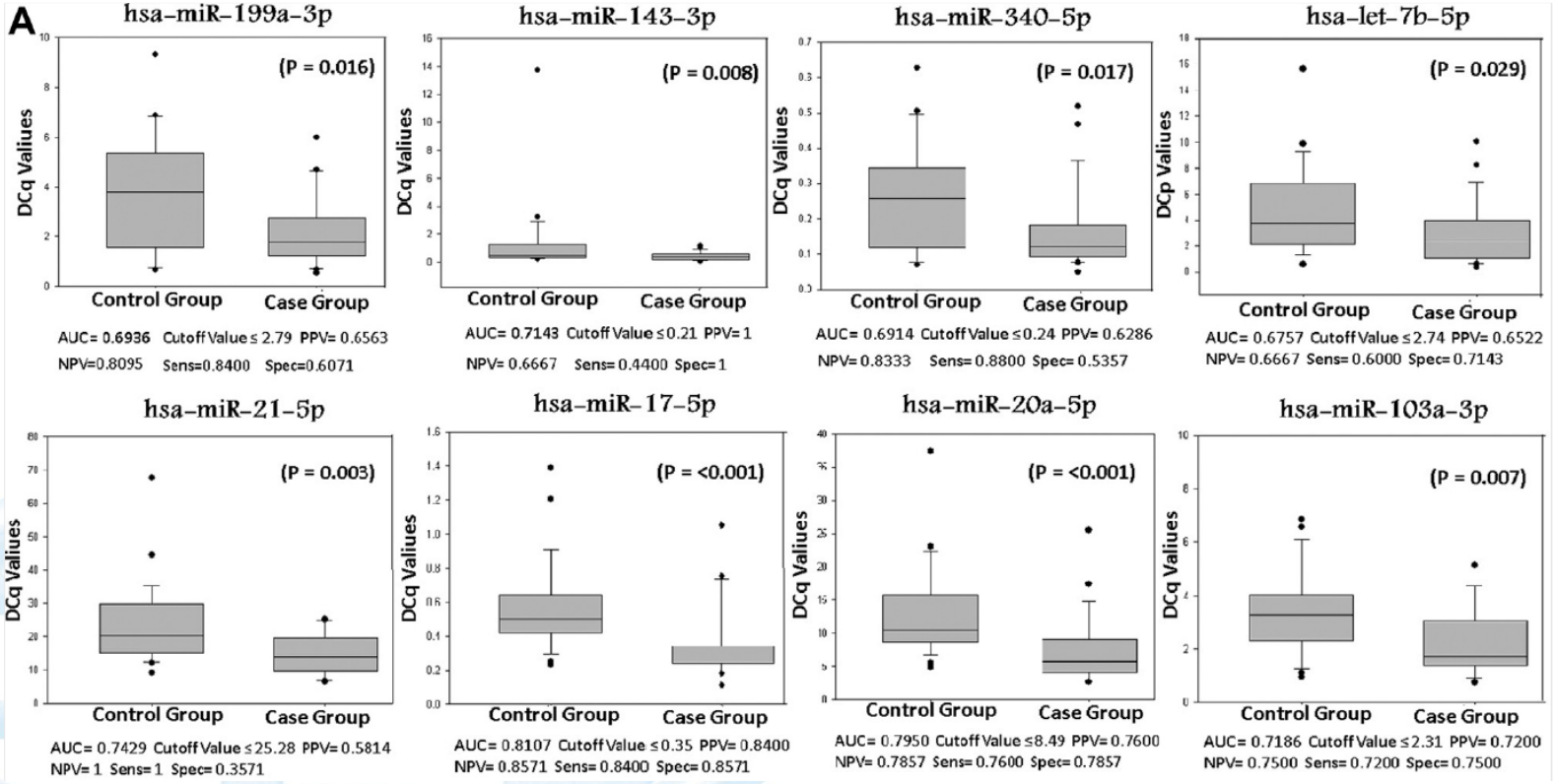
Papari et al. Fertil Steril 2020

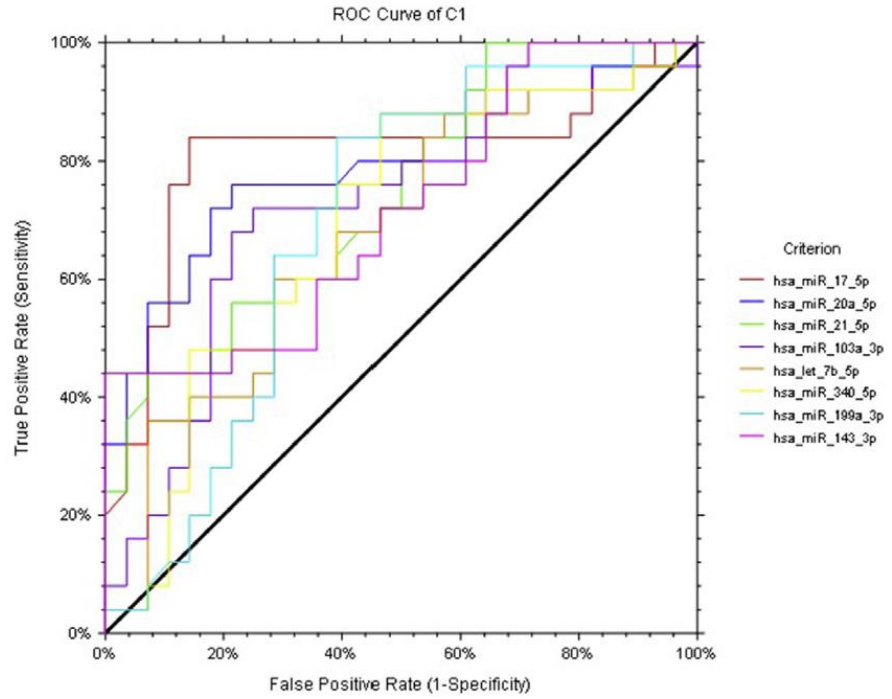
- ✓ Plasma
- ✓ Exploratory study (n=20)
  - Endo (n=10) & Control (n=10)
  - NGS
- ✓ Validation study (n=40)
  - Endo (n=25) – Control (n=15)
  - RT-qPCR
- ✓ Diagnostic laparoscopy because of pain, infertility, pelvic mass, myomas

Identification of candidate microRNA markers of endometriosis with the use of next-generation sequencing and quantitative real-time polymerase chain reaction

Elahe Papari, M.Sc.,[a] Mehrdad Noruzinia, M.D., Ph.D.,[a] Ladan Kashani, M.D.,[b] and Warren G. Foster, Ph.D.[c]

Papari et al. Fertil Steril 2020





Variability in endometriosis RNA biomarker research

✓ Biological

- Varibility introduced by differences in cases/control selection
- Disease heterogeneity (phenotype, symptoms, etc.), control group (symptomatic, laparoscopy,…)

✓ Pre-analytical

- Variability introduced by differences related to sample collection, processing & storage
- Serum vs plasma, hemolysis, biobanking method, etc

✓ Analytical

- Variability introduced by factors directly connected to the analytical method
- Profiling platform/array, data normalisation, etc.

Conclusion

- ✓ Biological link between endometriosis and certain miRNAs?
  - Plausible
  - Interesting for pathophysiology
- ✓ Only few studies include validation cohorts
  - Important drop in diagnostic perfomance
  - Limited overlap & conflicting data
- ✓ miRNAs as biomarkers for endometriosis?
  - We have a few ‘potential targets’…but a lot of work to do
  - Molecular stability does not exclude variability

Hot off the press…

scientific reports

Check for updates

OPEN

MicroRNome analysis generates a blood-based signature for endometriosis

Sofiane Bendifallah[1,2✉], Yohann Dabi[1,2,3], Stéphane Suisse[2], Ludmila Jornea[4], Delphine Bouteiller[5], Cyril Touboul[1,2], Anne Puchar[1,2] & Emile Daraï[1,2]

diagnostics

MDPI

Article

Endometriosis Associated-miRNome Analysis of Blood Samples: A Prospective Study

Sofiane Bendifallah [1,2,*], Yohann Dabi [1,2], Stéphane Suisse [3], Léa Delbos [4], Mathieu Poilblanc [5], Philippe Descamps [4], Francois Golfier [5], Ludmila Jornea [6], Delphine Bouteiller [7], Cyril Touboul [1,2], Anne Puchar [8] and Emile Daraï [1,2]

Journal of *Clinical Medicine*

MDPI

Article

Salivary MicroRNA Signature for Diagnosis of Endometriosis

Sofiane Bendifallah [1,2,*], Stéphane Suisse [3], Anne Puchar [1,2], Léa Delbos [4,5], Mathieu Poilblanc [6,7], Philippe Descamps [4,5], Francois Golfier [6,7], Ludmila Jornea [8], Delphine Bouteiller [9], Cyril Touboul [1,2], Yohann Dabi [1,2] and Emile Daraï [1,2]

International Journal of *Molecular Sciences*

MDPI

Article

A Bioinformatics Approach to MicroRNA-Sequencing Analysis Based on Human Saliva Samples of Patients with Endometriosis

Sofiane Bendifallah [1,2,3,*], Yohann Dabi [1,2,3], Stéphane Suisse [4], Ludmila Jornea [5], Delphine Bouteiller [6], Cyril Touboul [1,2,3], Anne Puchar [1] and Emile Daraï [1,2]

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Table 1. Demographic Characteristics of the population.

	Control Patients N (%) N = 47	Patients with Endometriosis N (%) N = 153	
Age (years) (mean ± SD)	30.92 (13.79)	31.17 (10.78)	0.1912
Age range			
- Less than 30 years	72% (34)	63% (96)	
- Over 30 years	28% (13)	37% (57)	0.294
BMI (body mass index) (mean ± SD)	24.84 (11.10)	24.36 (8.38)	0.525
Infertility			
- Yes	17% (8)	24% (36)	
- No	83% (39)	76% (117)	0.556
rASRM classification			
- I–II	-	52% (80)	-
- III–IV	-	48% (73)	
Control diagnoses			
- No abnormality	51% (24)	_	-
- Leiomyoma	2% (1)		
- Cystadenoma	11% (5)		
- Teratoma	23% (11)		
- Other gynecologic disorders	13% (6)		
Dysmenorrhea	100%	100%	
Abdominal pain outside menstruation			
- Yes	66% (21)	71% (89)	0.6905
Pain suggesting sciatica			
- Yes	31% (10)	56% (70)	0.0214
Lower back pain outside menstruation			
- Yes	62% (20)	81% (101)	0.0498
Right shoulder pain during menstruation			
- Yes	9% (3)	21% (26)	0.2184
Blood in the stools during menstruation			
- Yes	12% (4)	24% (30)	0.2425
Blood in urine during menstruation			
- Yes	25% (8)	17% (21)	0.4172
Diagnostic method			
- Surgery	47 (100)	83 (54.2)	
- Magnetic Resonance Imaging	-	70 (45.8)	-

Journal of *Clinical Medicine*

MDPI

Article

Salivary MicroRNA Signature for Diagnosis of Endometriosis

Sofiane Bendifallah [1,2,*], Stéphane Suisse [3], Anne Puchar [1,2], Léa Delbos [4,5], Mathieu Poilblanc [6,7], Philippe Descamps [4,5], Francois Golfier [6,7], Ludmila Jornea [8], Delphine Bouteiller [9], Cyril Touboul [1,2], Yohann Dabi [1,2] and Emile Daraï [1,2]

Table 2. Random Forest accuracies for endometriosis diagnosis.

	Random Forest		
Dataset	AUC	Sensitivity	Specificity
1	0.935	0.871	1
2	0.903	0.806	1
3	0.935	0.871	1
4	0.983	0.967	1
5	0.867	0.833	0.9
6	0.968	0.935	1
7	0.919	0.839	1
8	0.935	0.871	1
9	0.933	0.967	0.9
10	0.9	0.8	1

In yellow, the most accurate model.

Signature of 109 salivary miRNAs

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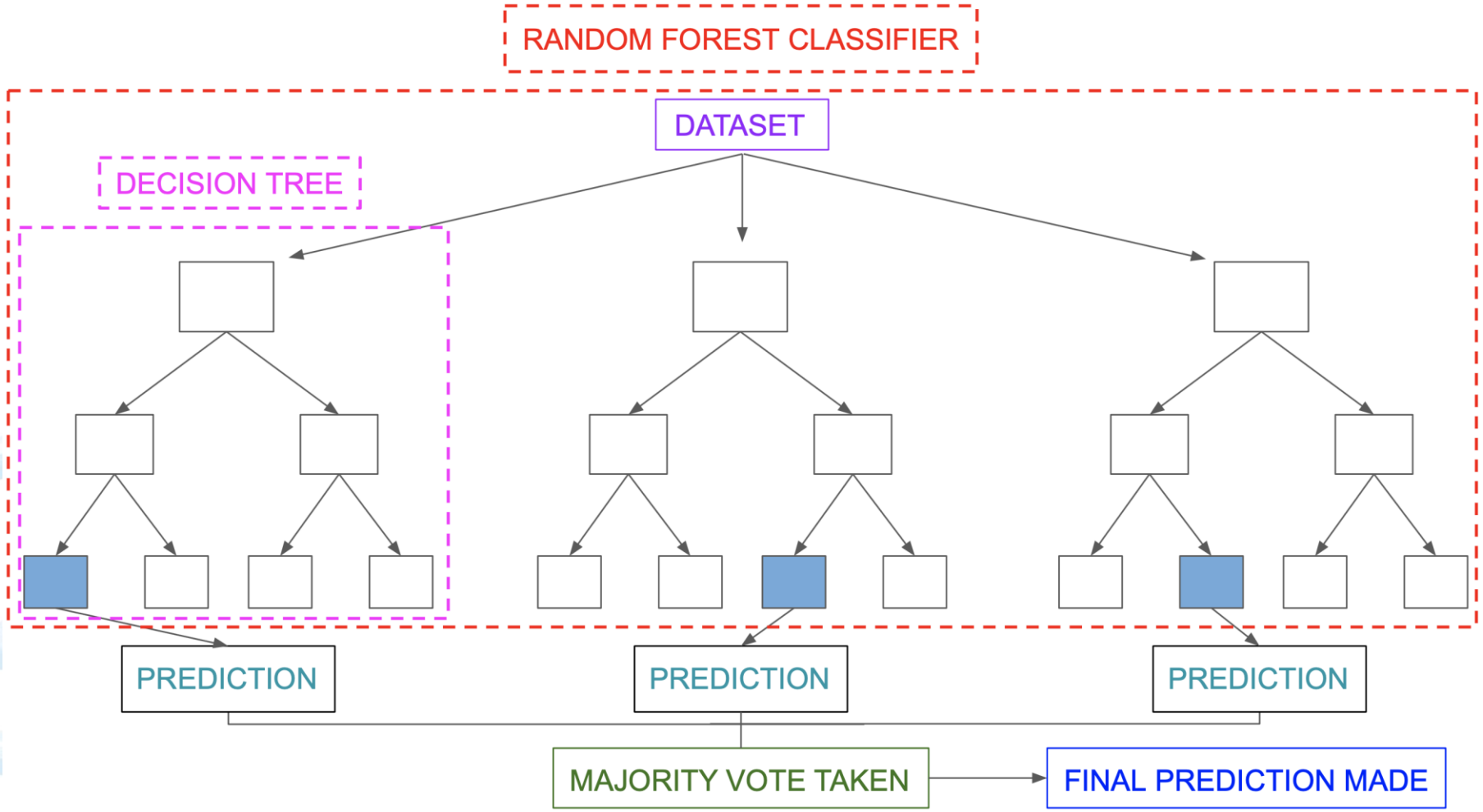
Random forest vs Decision tree



Challenges & limitations

- **Salami slicing**
  - 4 papers from same cohort…
- **Saliva**
  - Origin of salivary miRNAs?
- **Statistical "overfitting"**
  - Small sample size (47 controls…)

Test is now online available at a significant cost…

➔Independent validation needed!

➔Clinical application of test: performance in imaging negative patients?

Multicenter Validation of the Salivary miRNA Signature of Endometriosis

ClinicalTrials.gov Identifier: NCT05244668

Recruitment Status : Recruiting
First Posted : February 17, 2022
Last Update Posted : April 28, 2022

See Contacts and Locations

View this study on Beta.ClinicalTrials.gov

Sponsor:
ZIWIG

Collaborators:
Monitoring Force Group
iGenSeq

Information provided by (Responsible Party):
ZIWIG

Study Description

Go to

Brief Summary:

ENDOmiARN Salive Test is a multicentre external validation study of a salivary signature of endometriosis carried out in France in Obstetrics and Reproductive Medicine departments, in order to evaluate its performance and discuss its use in clinical practice. The clinical application is to significantly reduce the time to diagnosis and improve the care pathway for endometriosis.


To be continued...