Endometriosis Biomarkers

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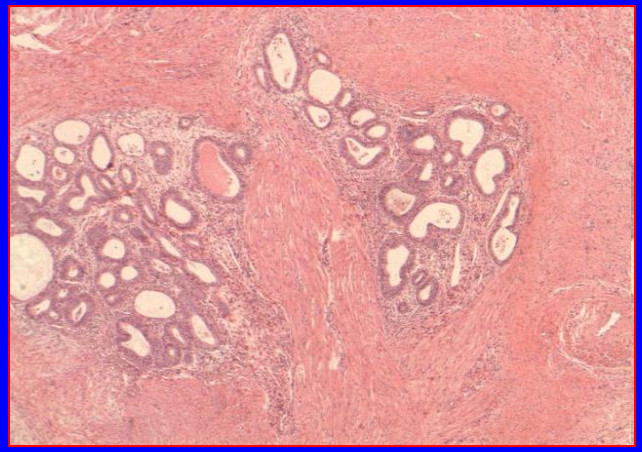
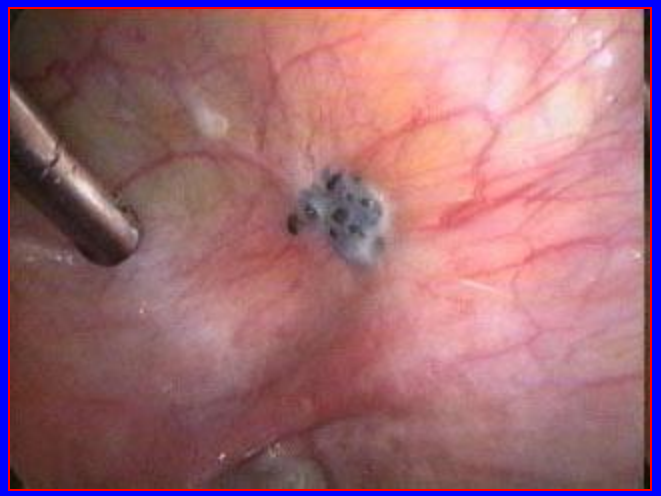
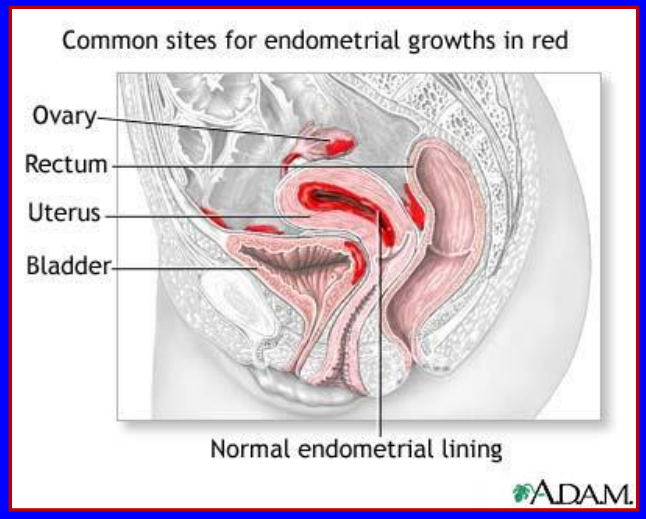
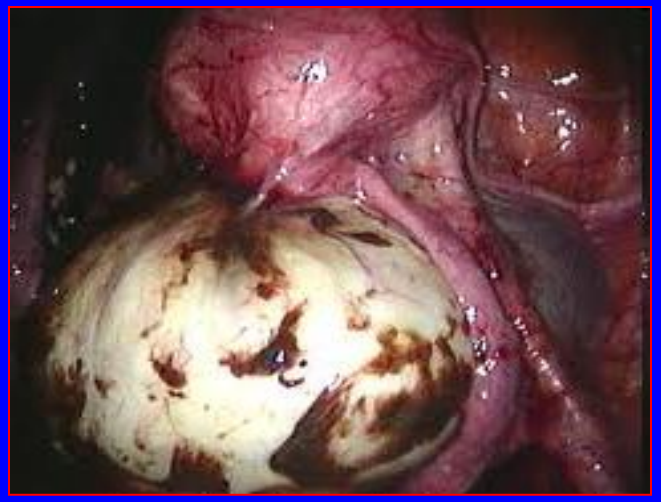
Endometriosis is Ectopic Endometrial Glands and Stroma









Clinical Presentation

- **Pain**
- **Infertility**
- **Asymptomatic**

Symptoms Are Not Related to Disease Stage

Percentage at Each Stage

	Stage				
	I	II	III	IV	*P*
Dysmenorrhea	73	86	72	85	.68
Pelvic pain	38	46	36	41	.21
Dyspareunia	30	25	36	29	.91

Fedele L et al. *Fertil Steril*. 1990;53(1):155-158.

Challenges in diagnosing endometriosis

- Many patients and PCPs unaware of disease
- Symptoms are nonspecific or associated with other disorders
- Survey of n = 7,025 women
  - 65% misdiagnosed
  - 46% saw ≥ 5 MDs to get correct diagnosis
- 6.7-11 years from symptom onset to diagnosis and treatment
- Early diagnosis and treatment can reduce uncertainty, discomfort, disease progression, and later complications

(Ballard et al, 2006; Nnoaham et al 2011; May et al 2011; Rogers et al, 2013)

Current limitations in endometriosis diagnosis:

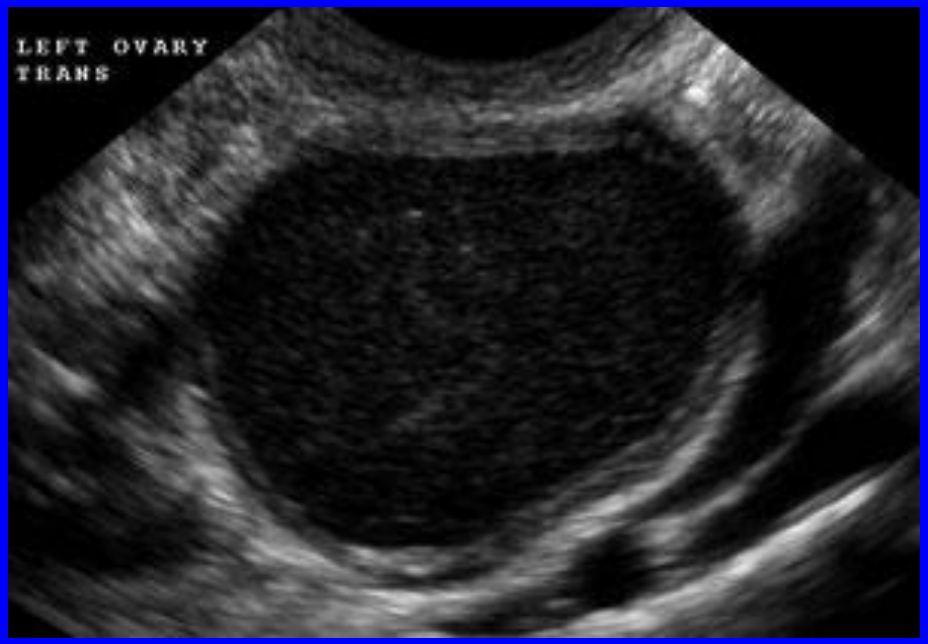
- No non-invasive diagnostic tests
- Requirement for surgical diagnosis is a barrier to timely diagnosis and therapy

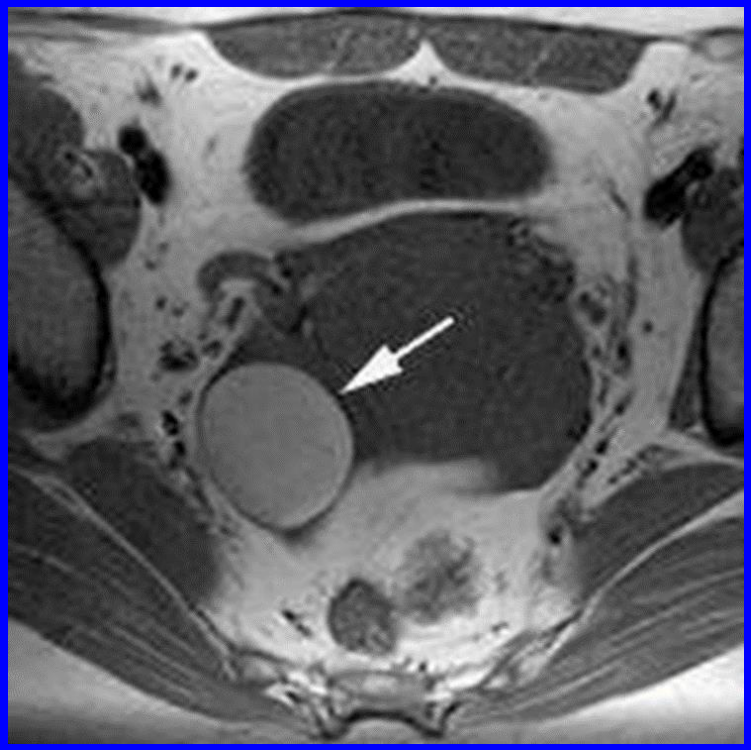
Clinical Presentation

Symptoms vary but typically reflect area of involvement and may include:

- Dysmenorrhea
- Cylical/noncylical pelvic pain
- Lower abdominal or back pain
- Dyschezia, often with cycles of diarrhea/constipation
- Bloating, nausea, and vomiting
- Inguinal pain
- Dysuria
- Dyspareunia
- Nodules may be felt upon pelvic exam
- Typically develops on pelvic structures, ie, rectovaginal septum, bladder, bowel, intestines, ovaries, and fallopian tubes
- Ovaries most common locations; gastrointestinal tract, urinary tract, soft tissues, and diaphragm follow
- Imaging may indicate pelvic mass/endometriomas.
- Less commonly found in distant regions, eg, diaphragm, lungs (inducing catamenial pneumothorax), and rarely, areas far outside abdominopelvic region

Endometriosis – U/S or MRI





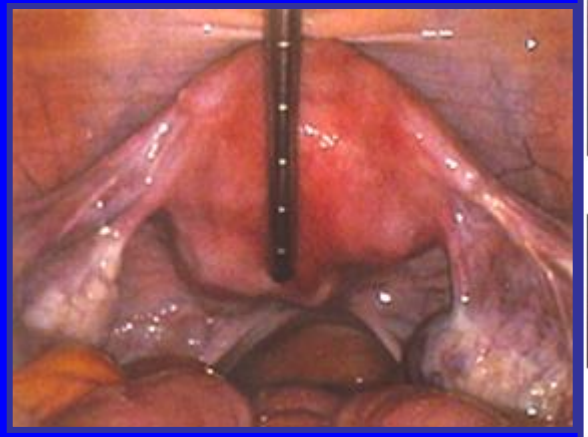
Laparoscopy/laparotomy

- Pros:
  - “Gold standard”
  - Can also remove lesions
- Cons
  - Invasive procedure
  - Has its own risk of morbility and, rarely, mortality
  - Costly
  - Still difficult to detect microscopic and/or subperitoneal lesions
  - Accuracy depends on the skill levels of surgeons

Surgical Diagnosis

Sites of disease

- Most common -
  - Peritoneum
  - Ovaries
  - Cul de sac
- Others
  - Pleural cavity
  - Bladder
  - Brain
  - Men



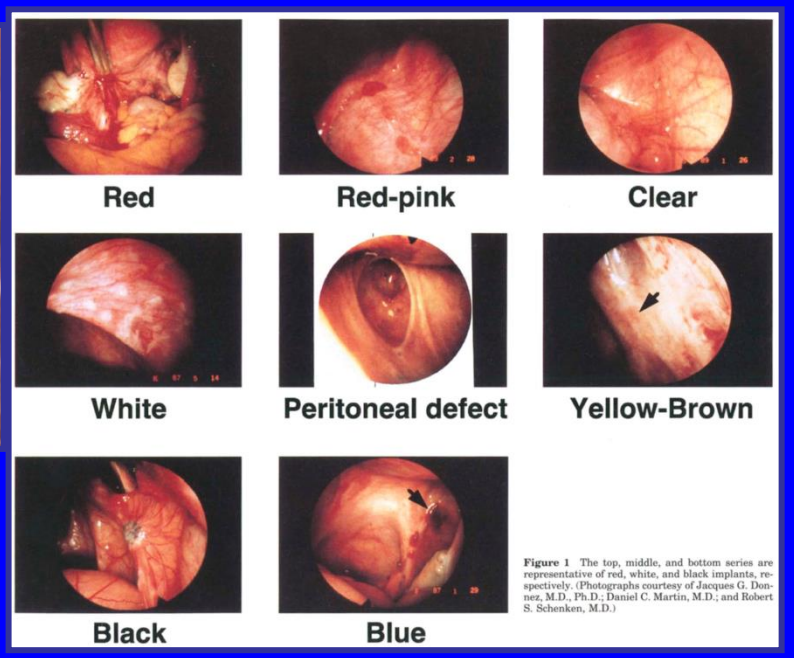
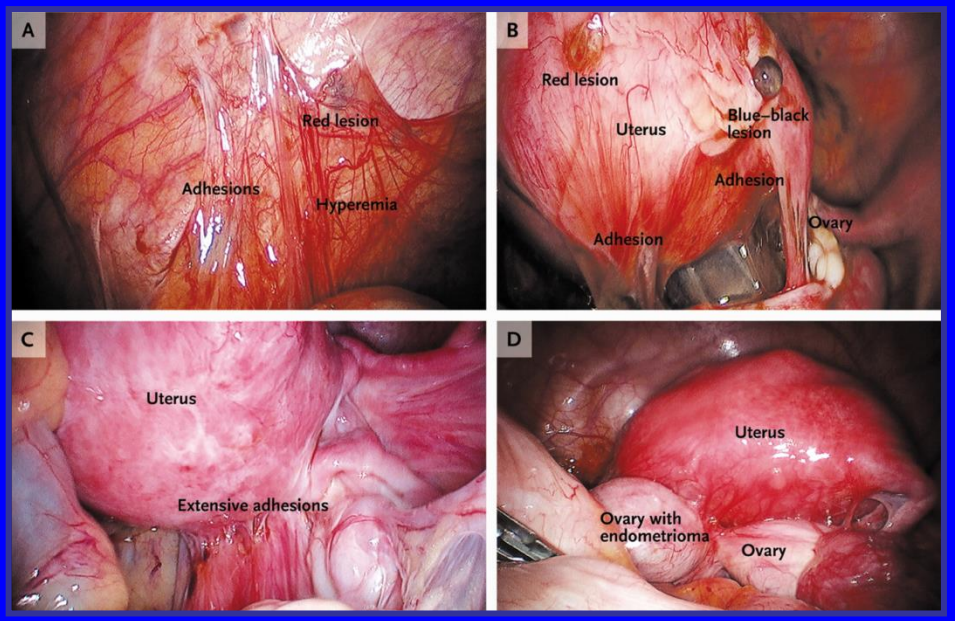


Figure 1 The top, middle, and bottom series are representative of red, white, and black implants, respectively. (Photographs courtesy of Jacques G. Donnez, M.D., Ph.D.; Daniel C. Martin, M.D.; and Robert S. Schenken, M.D.)



Subtleties in appearance can lead to misdiagnosis

Giudice L. *N Engl J Med.* 2010;*362*(25):2389–2398

Normal Peritoneum has Endometriosis in 25% of Women with PP

- **Unsuspected endometriosis documented by scanning electron microscopy in visually normal peritoneum.**

Murphy AA, Green WR, Bobbie D, dela Cruz ZC, Rock JA.
Fertil Steril. 1986;46(3):522-4

Only 45% of Visualized Endometriosis Confirmed by Histology

- A prospective study of 44 patients undergoing laparoscopy for the evaluation of chronic pelvic pain.
- The positive predictive value was 45%.

Walter AJ et al. Am J Obstet Gynecol. 2001;184(7):1407-11

Summary of Biomarker Uses:

- Risk assessment Biomarkers:
  - Identify those at risk for development of disease
  - Opportunity for monitoring or prophylactic therapy
- Diagnostic Biomarker:
  - Diagnostic clarity
  - Early diagnosis
  - Monitor asymptomatic, high-risk individuals

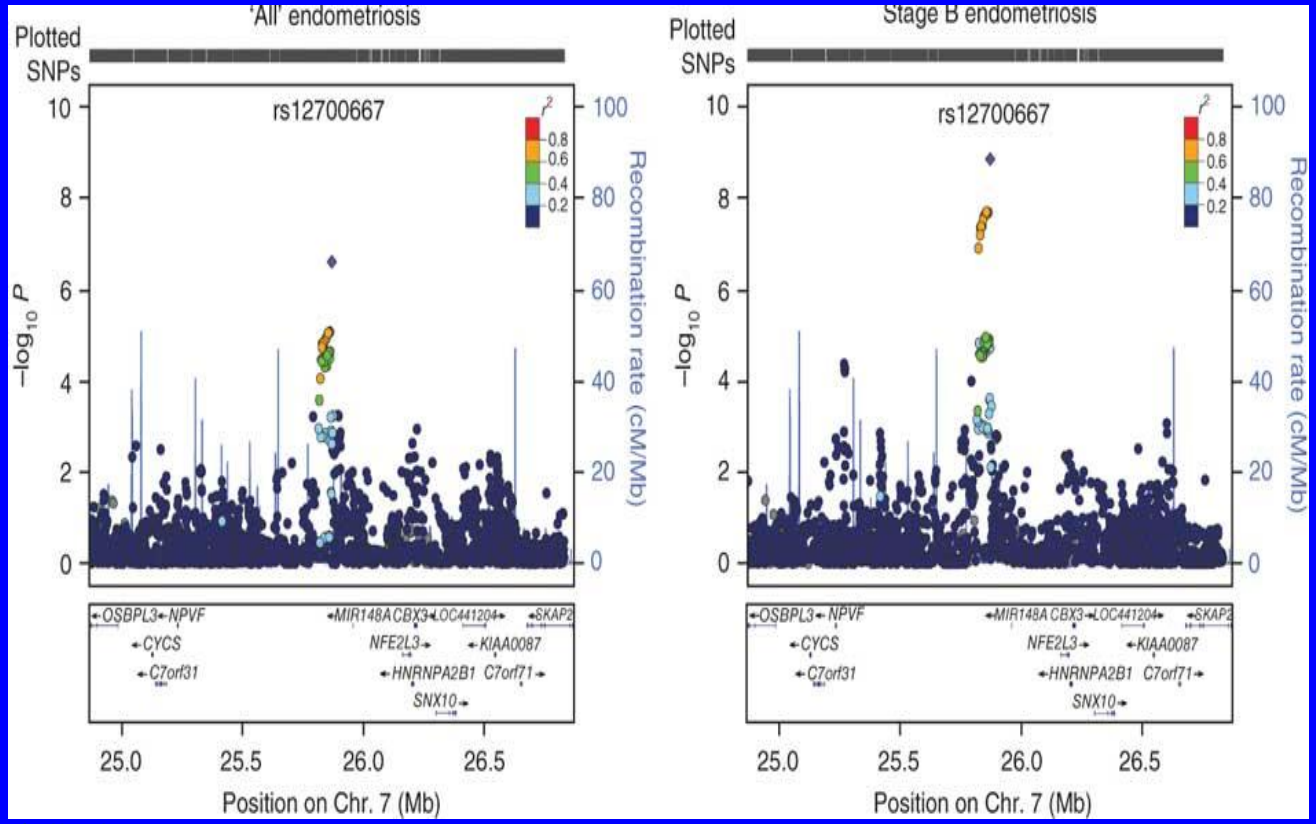
- **Prognostic Biomarker**
  - Predict disease outcome
  - Monitor disease recurrence
  - Predict disease free interval
- **Predictive Biomarker**
  - Choose optimal therapy
  - Monitor therapy response
  - Alter therapy in setting of emerging resistance

Types of biomarkers by clinical application

- Biomarkers of risk
- Biomarkers for early detection/diagnosis
- Disease outcomes: prognostic biomarkers
- Treatment outcome: predictive biomarkers

Risk Assessment Biomarkers

- Family History
- Exposures and Environmental Factors
- Genetics



Painter et al. Nature Genetics 2011;43:51–54

Genetic Tests

Exome sequencing

“Low-Frequency, Damaging Mutations in Hundreds of Genes Are Risk Factors for Endometriosis”

The ARTGuide Test

Predictive Technology Group and Juneau Biosciences

Society of Reproductive Investigation
Annual Meeting 2018

Types of biomarkers by clinical application

- Biomarkers of risk
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- Disease outcomes: prognostic biomarkers
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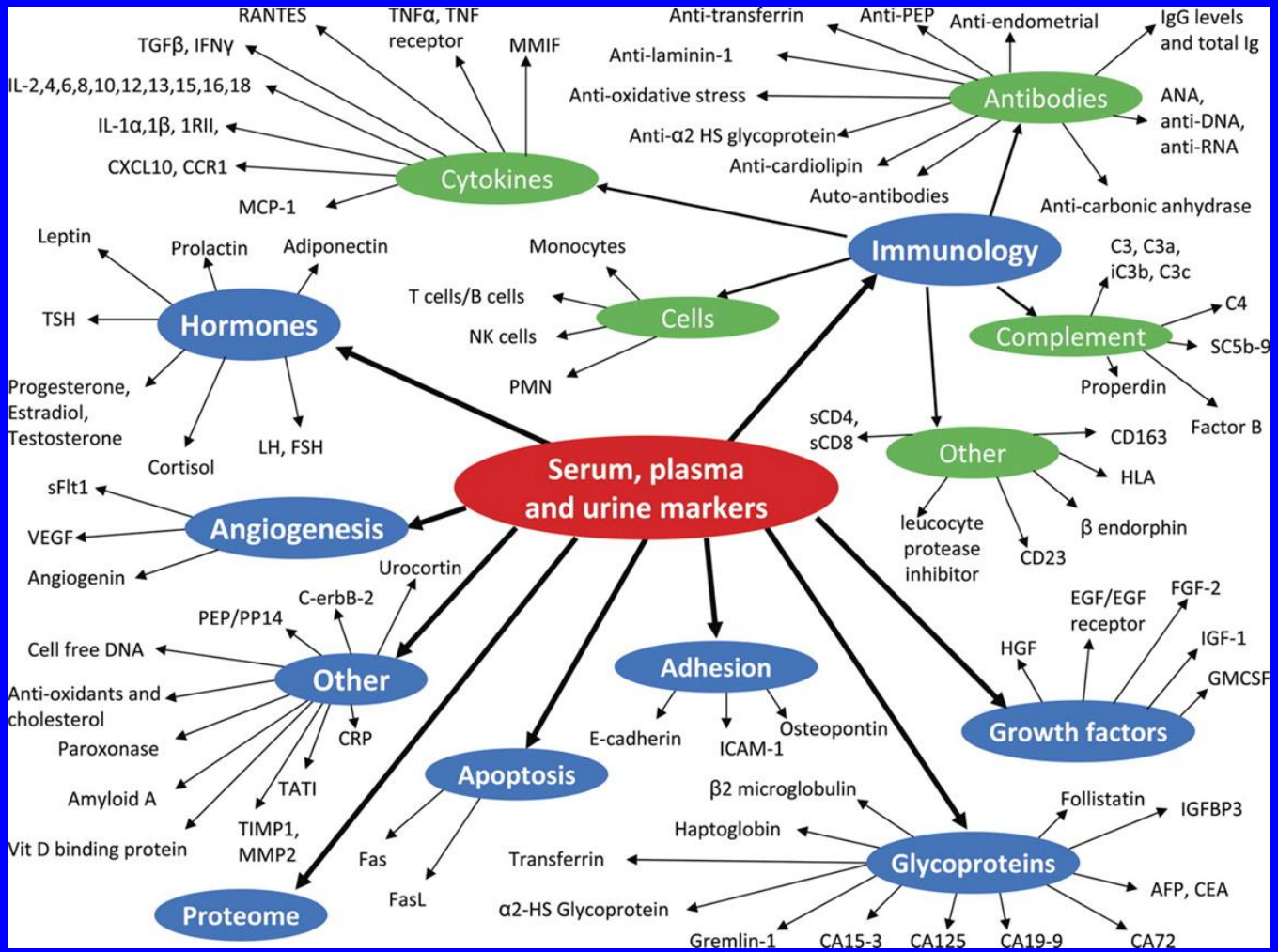
Classifiers Based on Endometrial Gene Expression

Diagnostic Variant	Cycle Phase	Construction Samples	Validation Samples	Cross-Validation Folds	Classifiers /Family	Validation Accuracy
Phase specific	PE	22	6	9	43	100%
	ESE	14	4	5	22	100%
	MSE	21	6	5	44	100%

Tamaresis et al, Molecular Classification of Endometriosis and Disease Stage Using High-Dimensional Genomic Data . Endocrinology. 2014; 155(12): 4986–4999

Serum Markers

- Over 200 different serum biomarkers have been proposed, yet none have adequate specificity and sensitivity.



Where are we now with biomarkers?

The most widely-used at present is CA-125, a marker that is inadequate on its own in terms of sensitivity and specificity

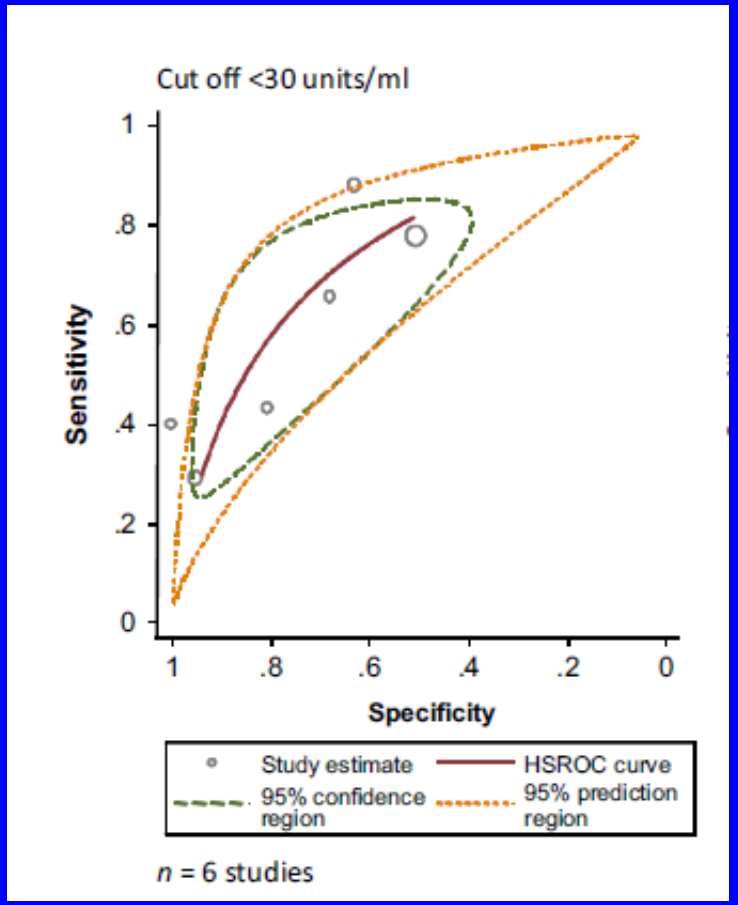
Biomarker	Sensitivity	Specificity
Anti-endometrial antibodies	81%	75%
IL-6	63%	69%
CA 19-9	36%	87%
CA 125 (low cutoff)	70%	64%
CA 125 (high cutoff)	50%	91%

CA-125

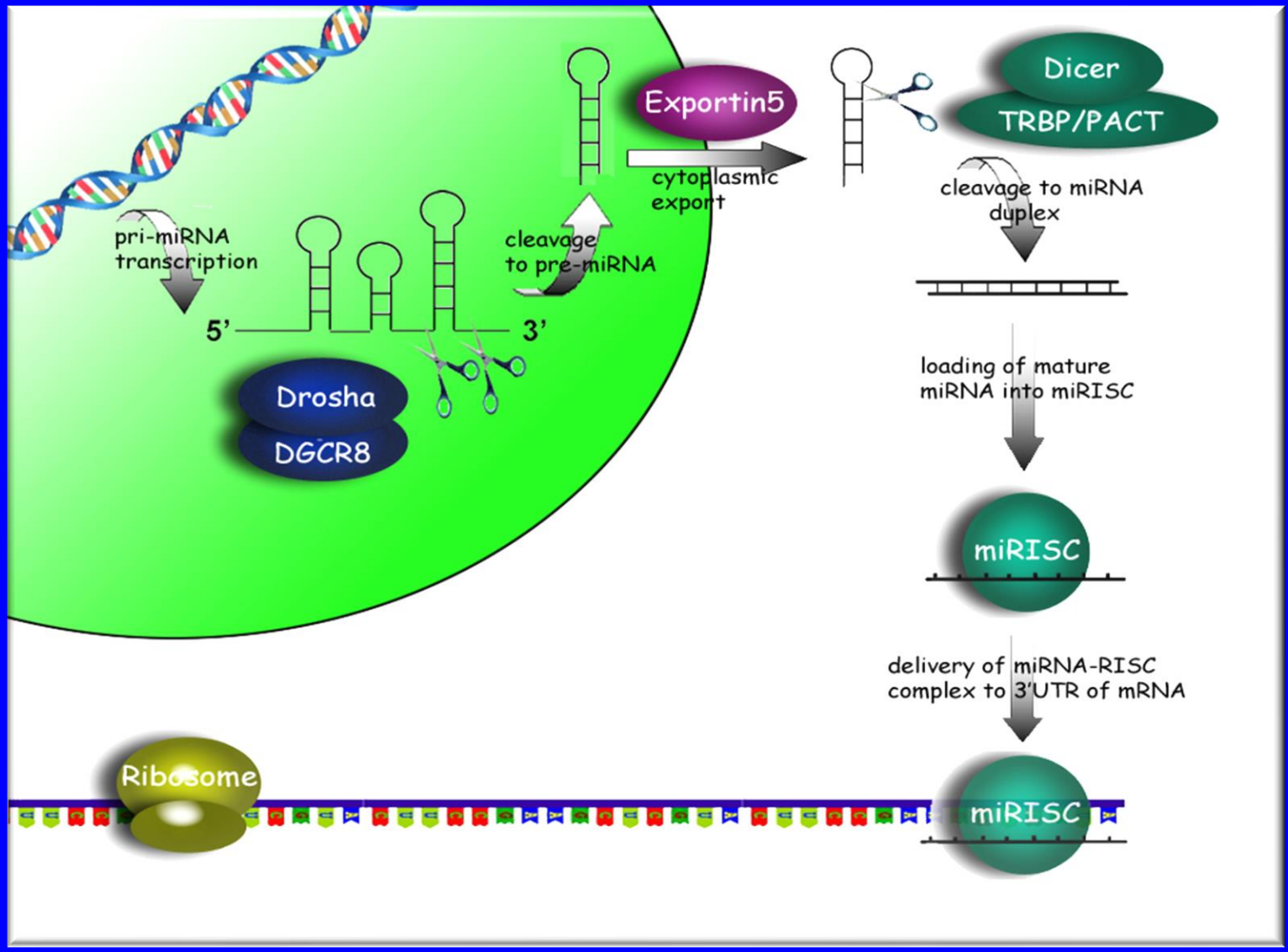
Medscape® www.medscape.com

Reference (*n* = Number of Patients)	Assay;Timing of Sample Collection	Stage	Sensitivity (%)	Specificity (%)
Barbieri et al, 1986[91]	Standard assay; timing of	All	17	96
(*n* = 147)	sample collection unknown	III+IV	54	96
Patton et al, 1986[177]	Standard assay; timing of	All	14	93
(*n* = 113)	sample collection unknown	III+IV	18	93
Pittaway and Fayez, 1986[92]	Standard assay (cutoff level	All	17	93
(*n* = 414)	30 IU/mL); follicular phase	III+IV	42	93
Koninckx et al, 1992[94]	Standard assay; late luteal	All	13	96
(*n* = 259)	phase	III+IV	31	94
O'Shaughnessy et al, 1993[96]	Standard assay; menstrual	All	27	100
(*n* = 100)		III+IV	67	100
Hornstein et al, 1995[97]	Standard assay; early	All	16	92
(*n* = 123)	follicular phase	III+IV	40	92
	CA 125 II assay; early	All	23	94
	follicular phase	III+IV	60	94
Medl et al, 1997[114]	Standard assay; timing of	All	36	92
(*n* = 368)	sample collection unknown	III+IV	44	86
Chen et al, 1998[107]	CA 125 II assay;	All	61	88
(*n* = 157)	luteal phase	III+IV	87	88

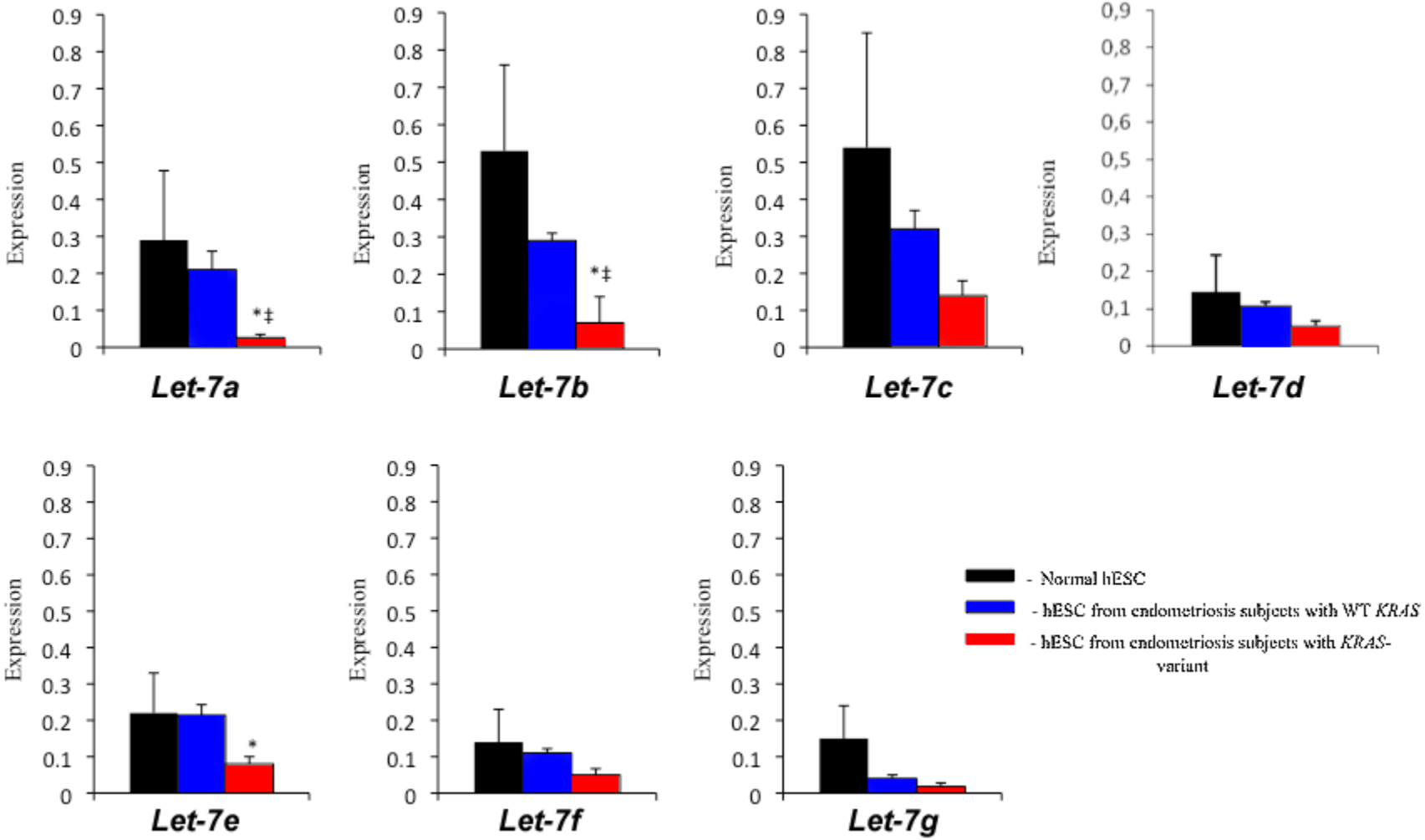
Source: Semin Reprod Med © 2003 Thieme Medical Publishers



Micro RNA (MiRNA)

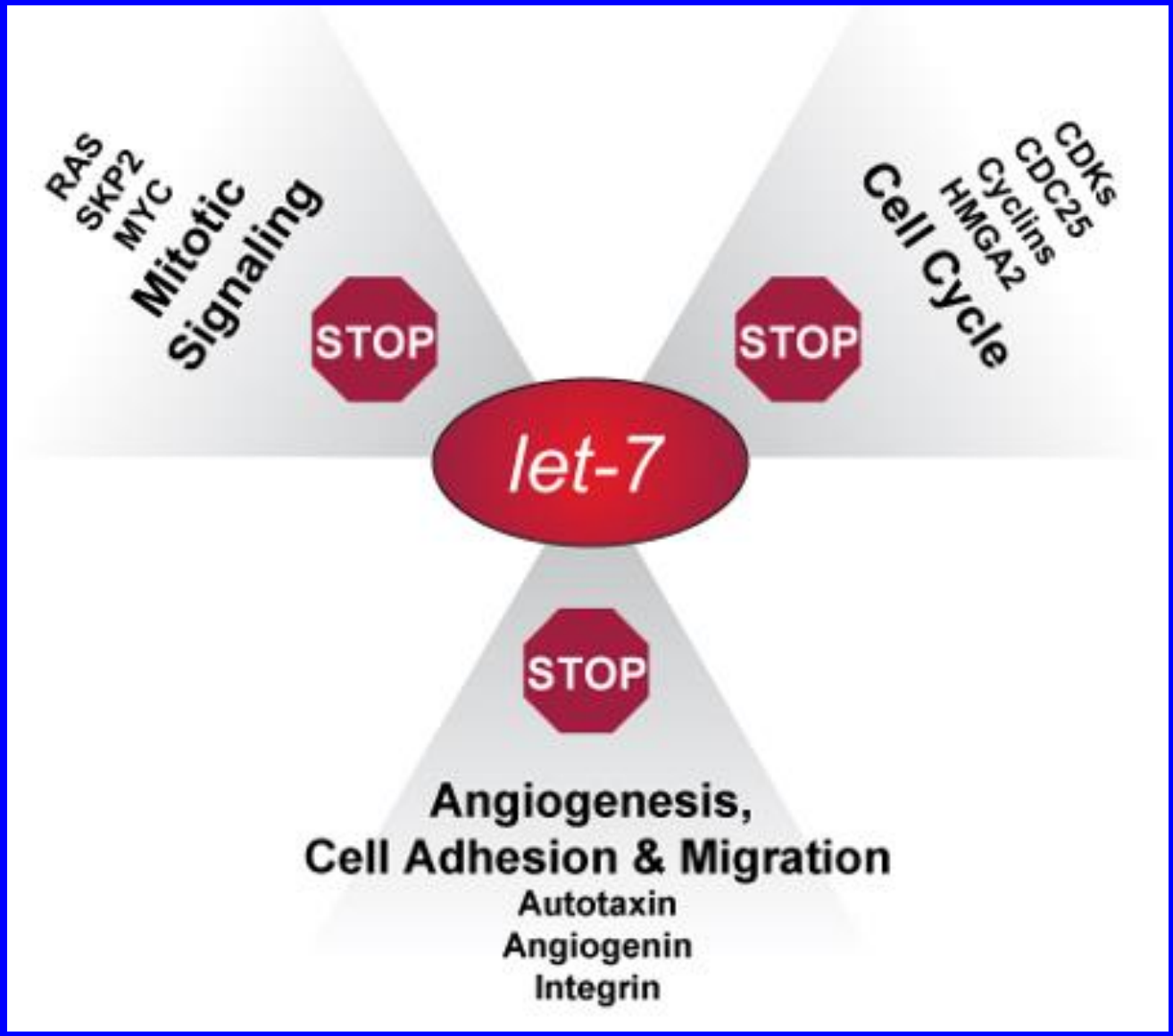


Decreased Let-7 micro RNAs in Endometriosis



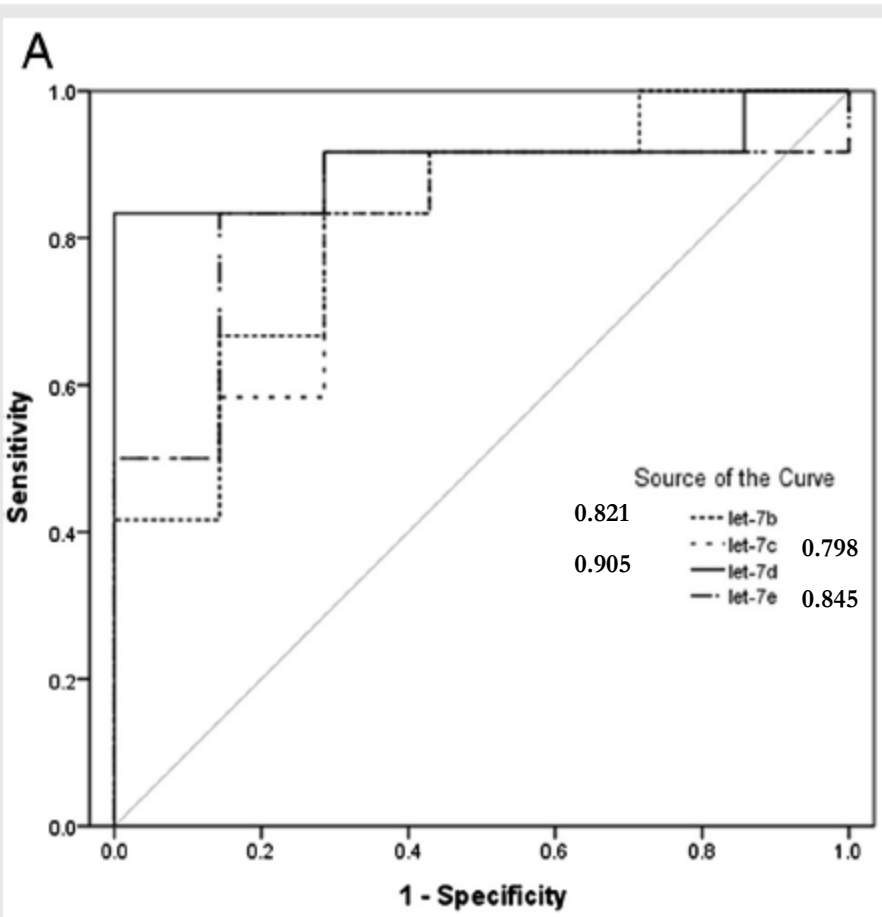
Grechukhina et al, EMBO Mol Med 2012;4(3):206-217

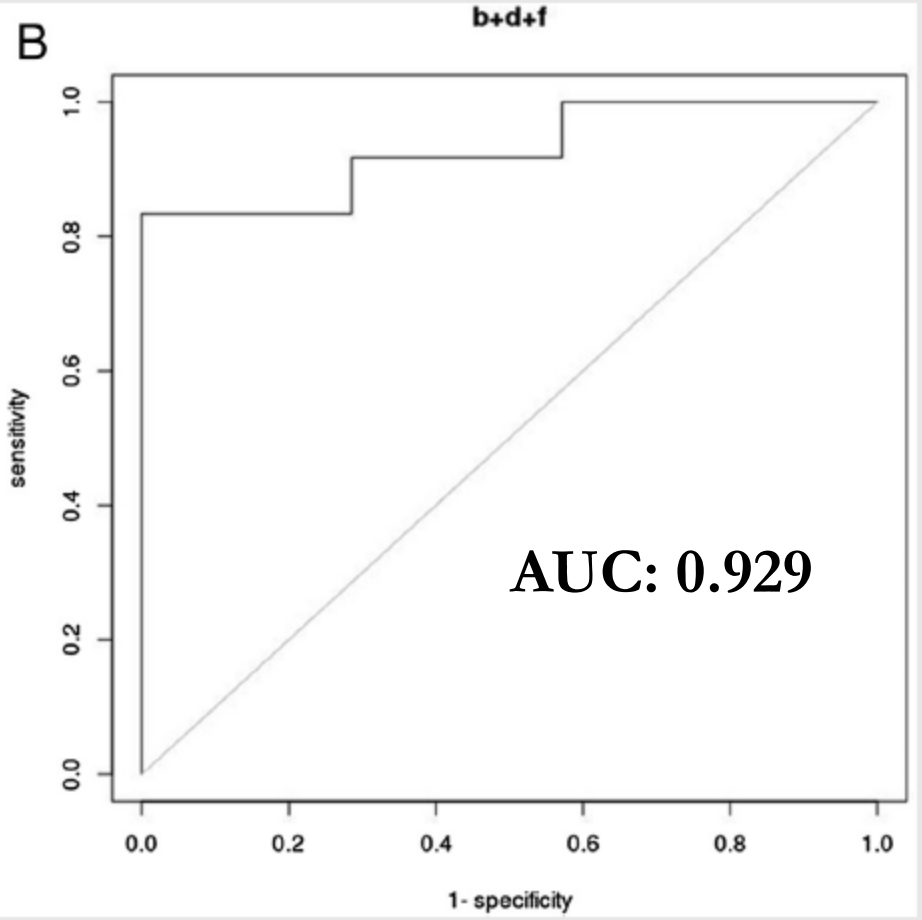
Let-7 signaling

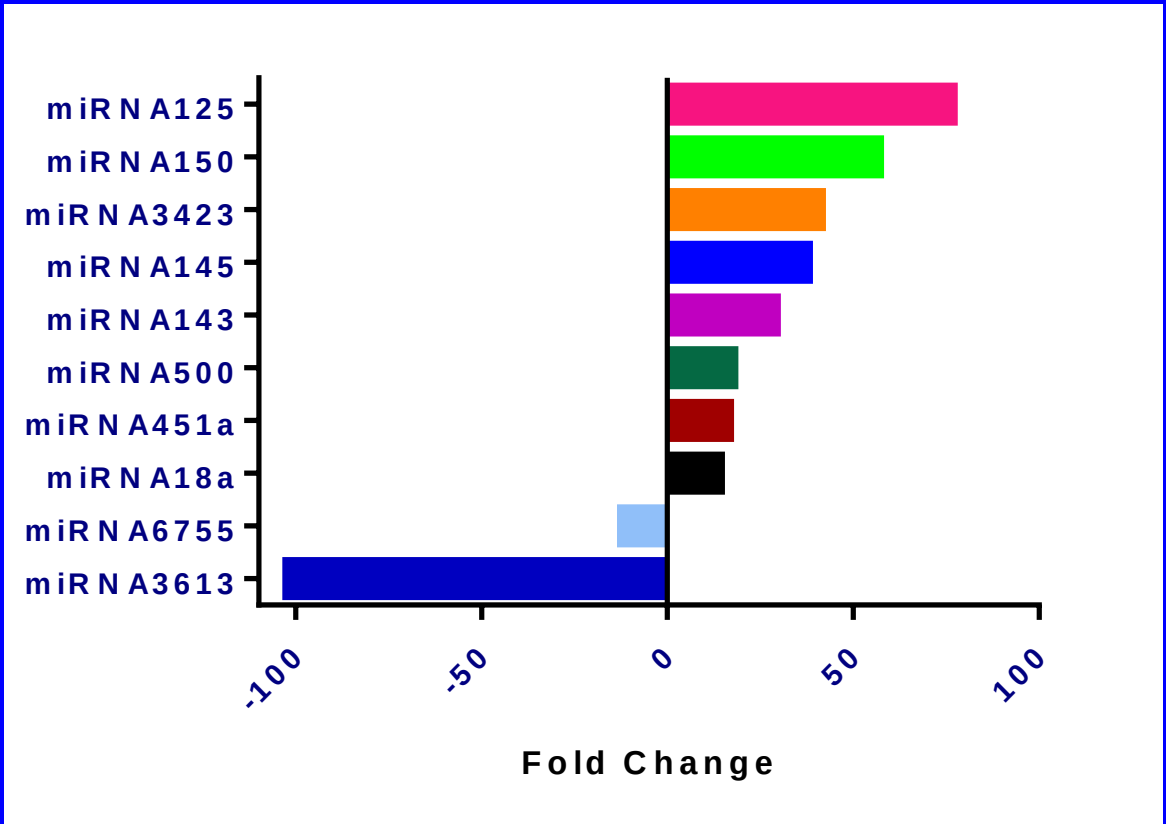


Circulating miRNAs as Serum Biomarkers of Endometriosis

ROC Curves of let7 During the Proliferative Phase of Menstrual Cycle



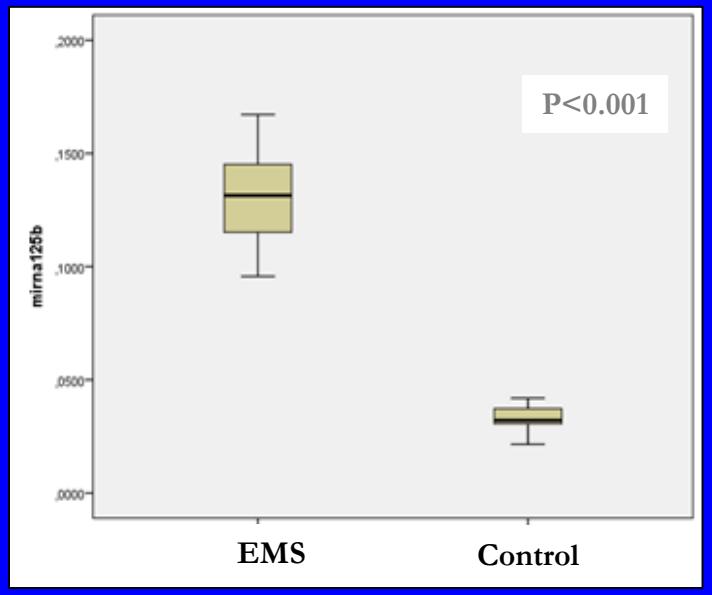




Cosar et al, Fertil Steril 2016

Serum miRNAs in Endometriosis

125b



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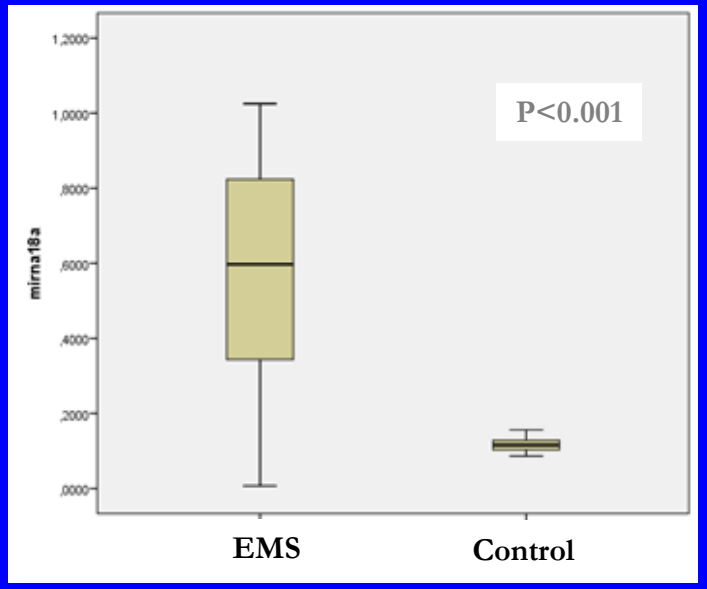

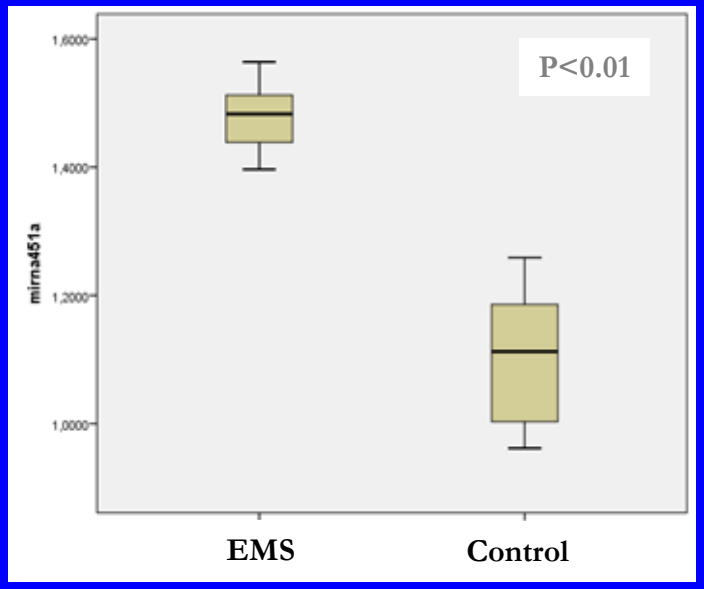

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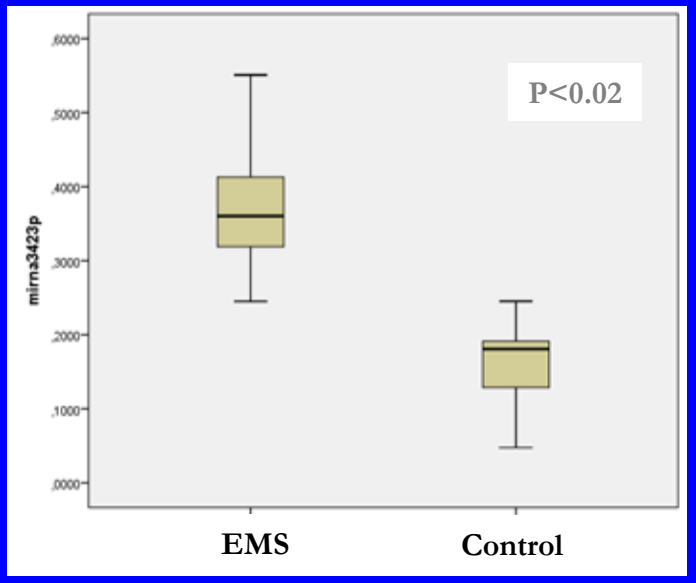

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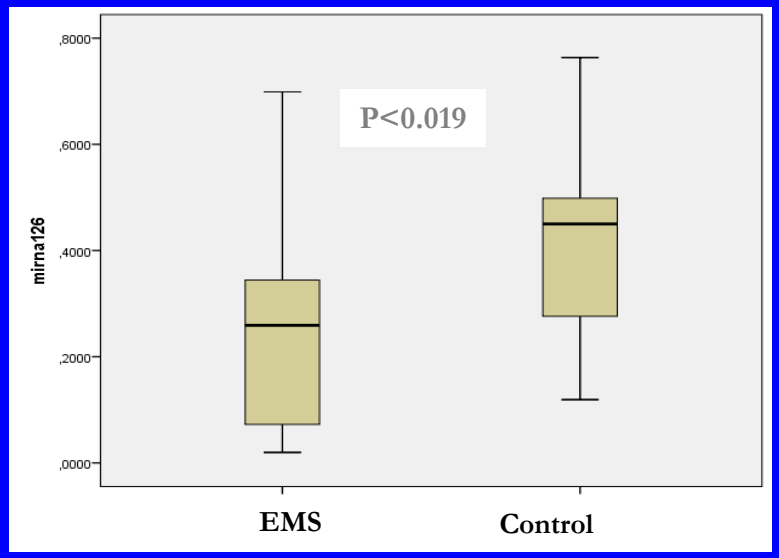
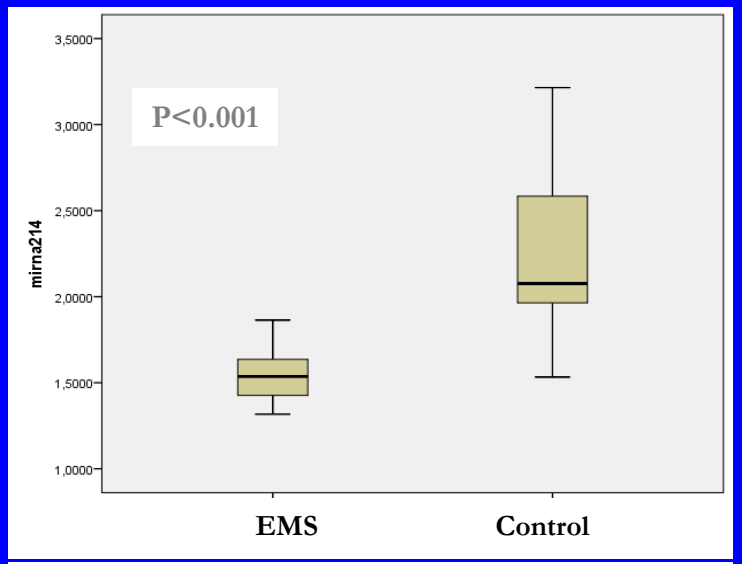
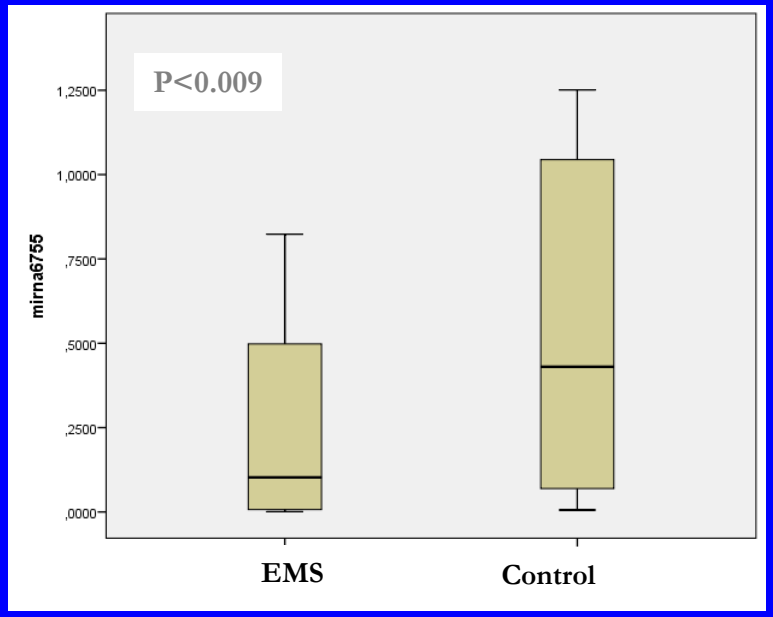
Down-regulated miRNAs in Endometriosis

126-3p

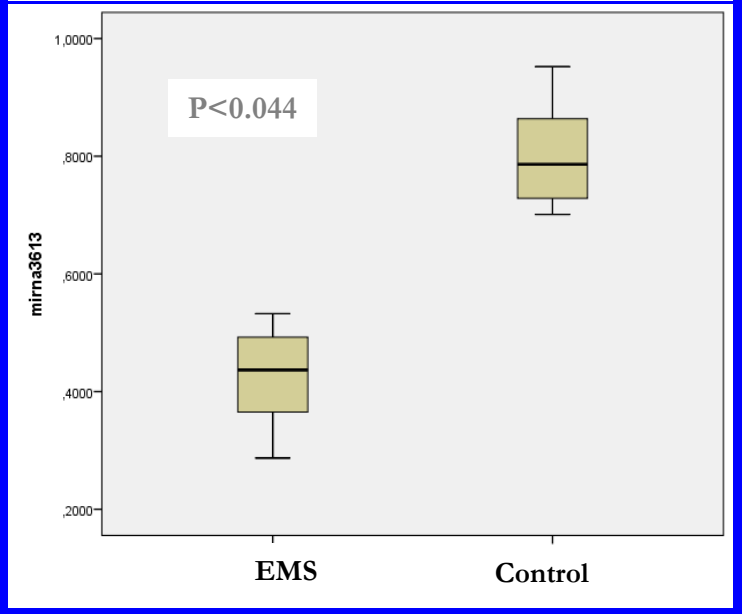


214-3p





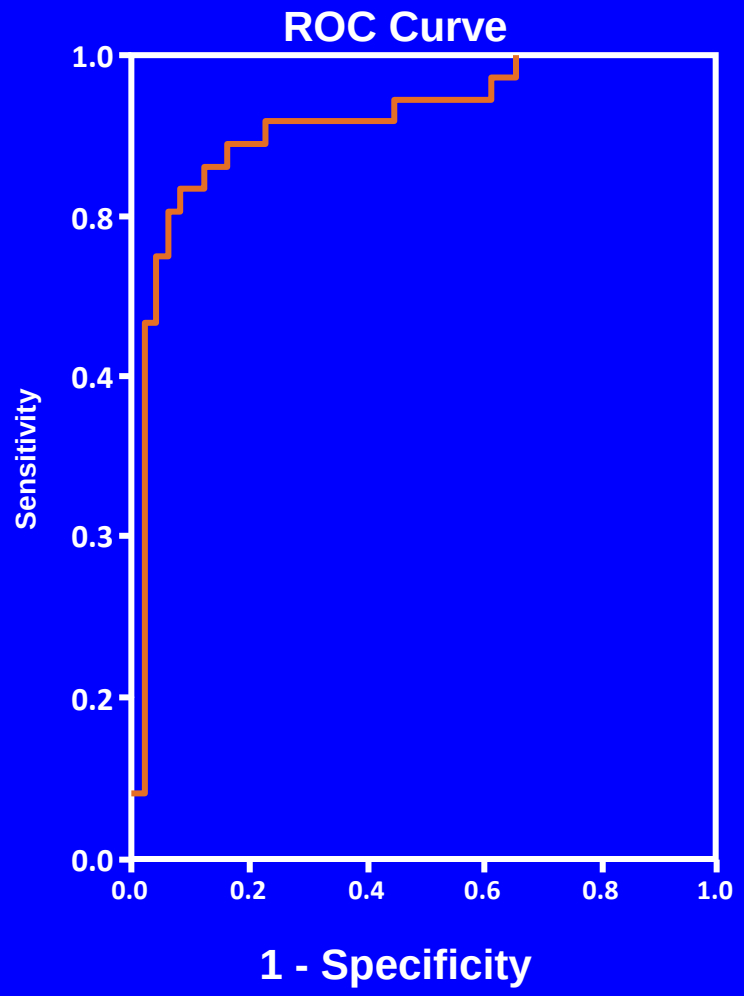
6755-3p



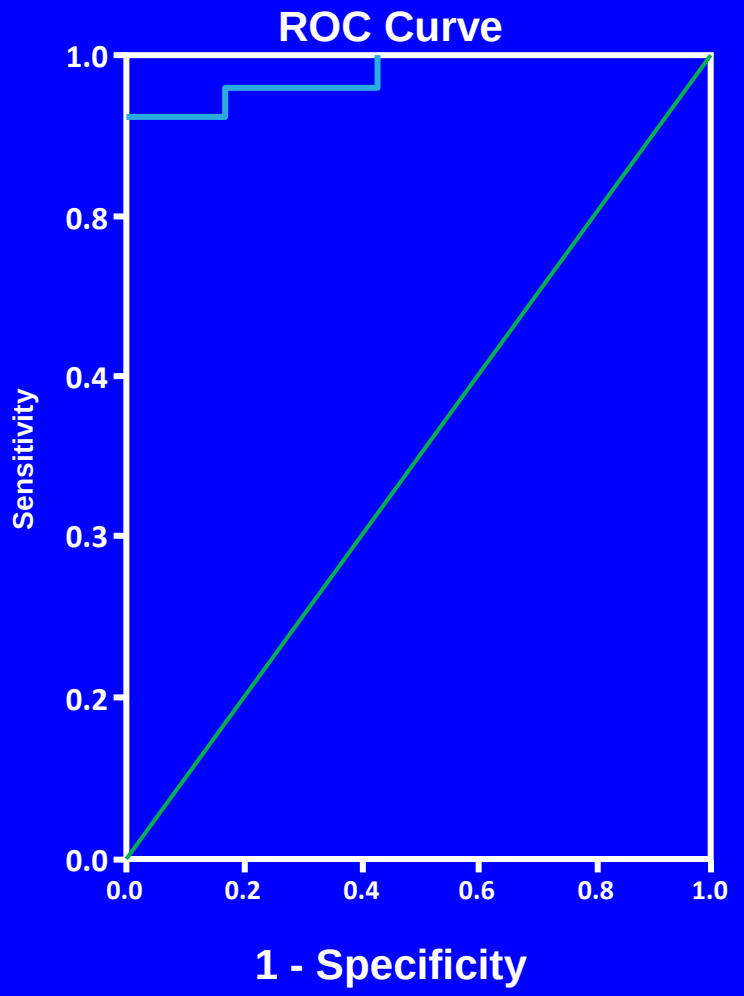
3613-3p

Combined miRNAs

MC_125_451_3613 AUC: 0.917

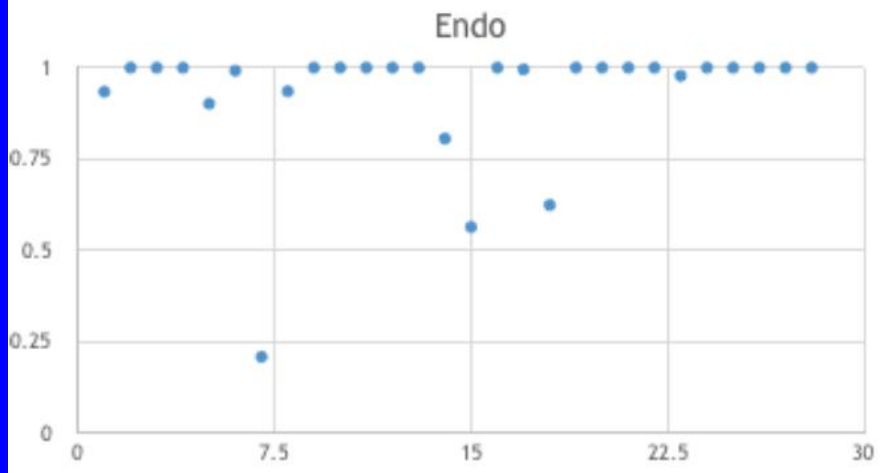


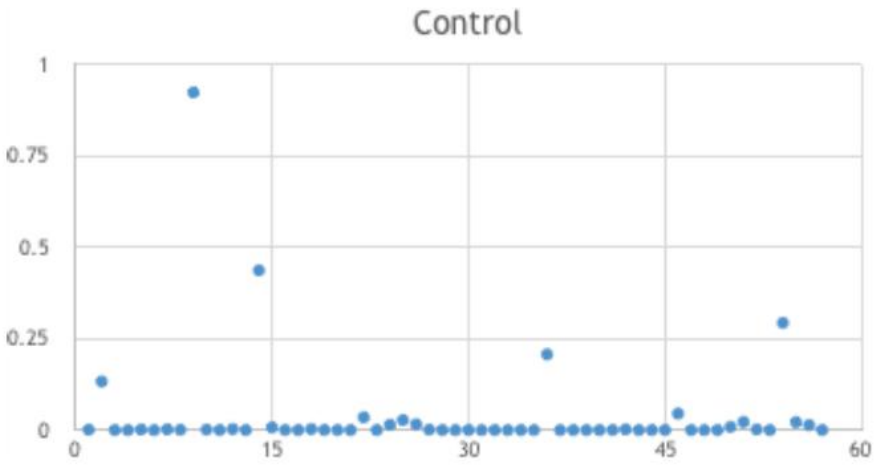
MC (125-let7b-150-342-451-3613) AUC: 0.977



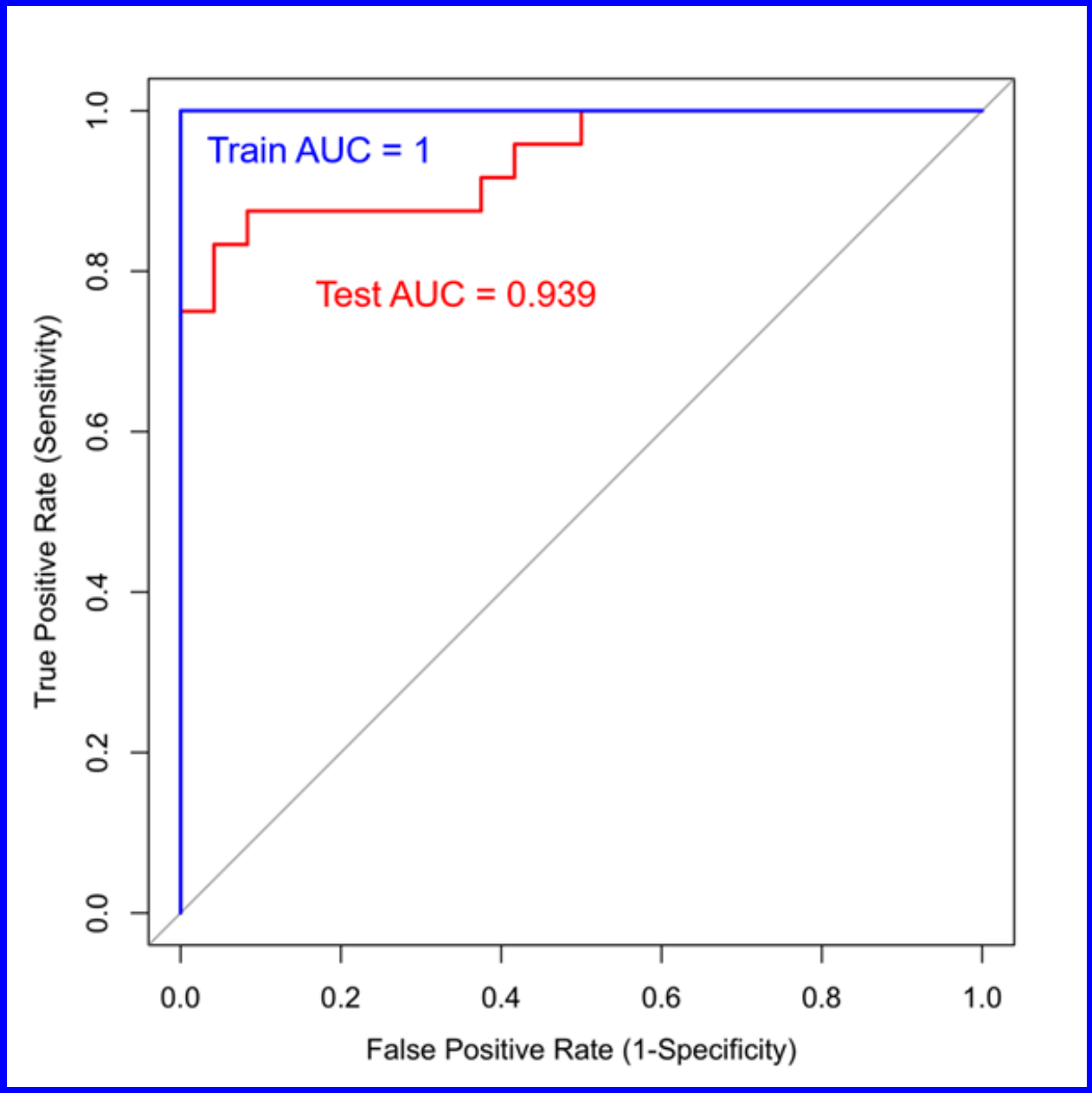
Mustafa S et al. Fertil Steril. 2017; 108: e44; Data presented at ASRM Scientific Congress, November 2017.

5 marker model





- Algorithm able to provide clear distinction between endometriosis and other benign gynecological pathologies
- Only 1 false positive and 1 false negative observed in above analysis of algorithm performance in prospective dataset



Mustafa et al; Am J OB GYN 2020

Response to Treatment Study

Healthy adult female baboons (N=16)

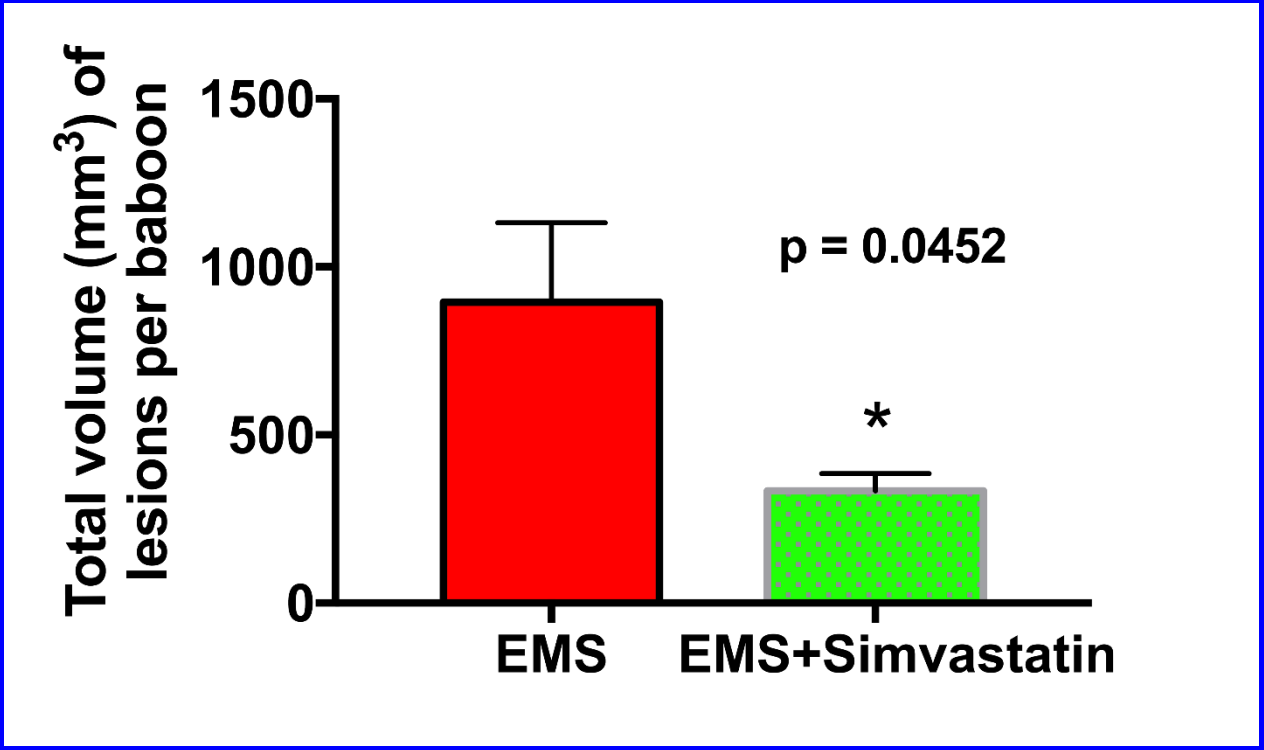
• Randomized into 2 groups (treatment group with 20 mg/day simvastatin and control)

• Endometriosis induced by laparoscopy using menstrual endometrium

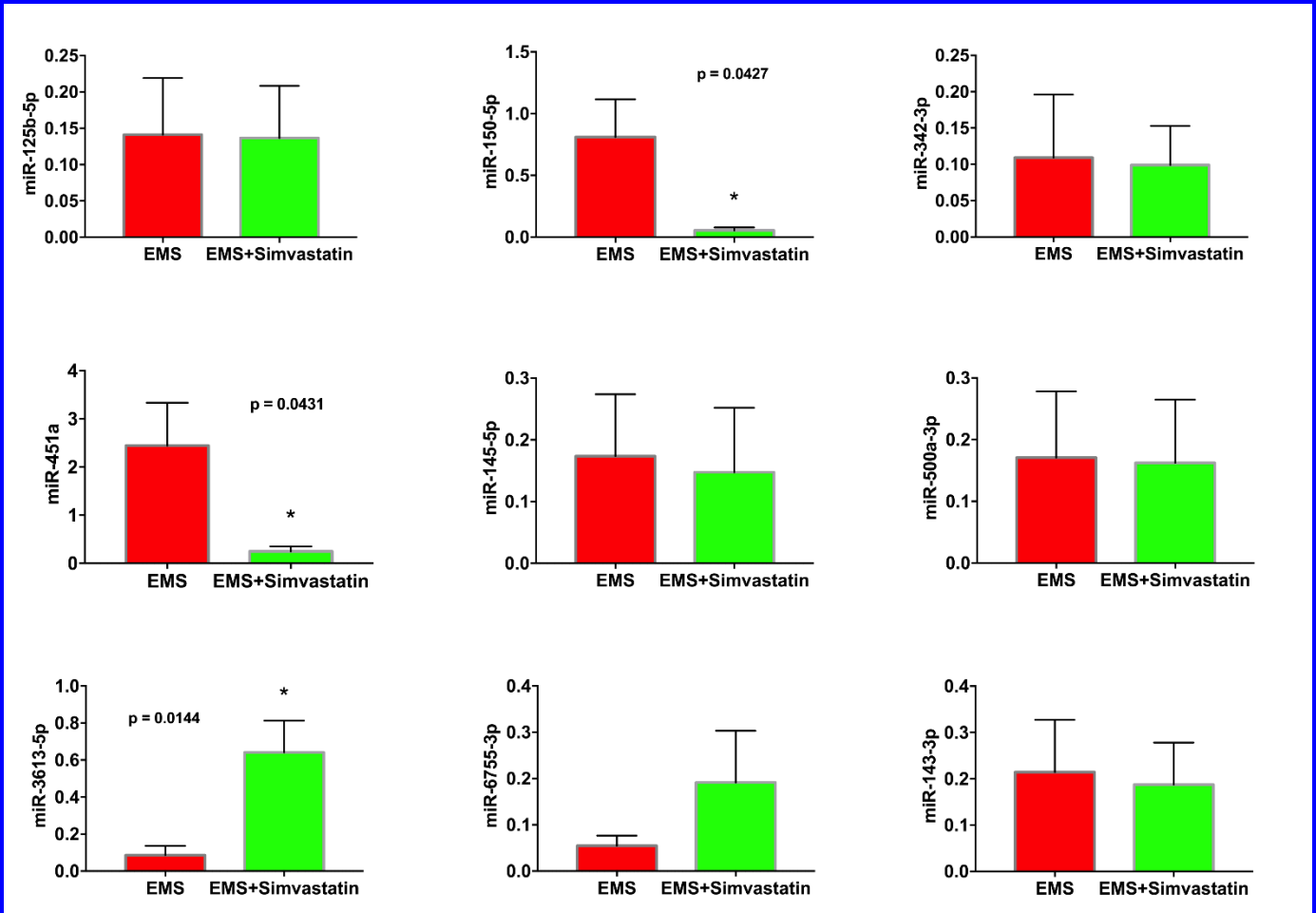
• Laparoscopy performed after 3 months to evaluate extent of disease and also measured miRNA biomarkers

Cosar et al Reprod Sci 2018

Treatment Response



MicroRNA response to therapy



Types of biomarkers by clinical application

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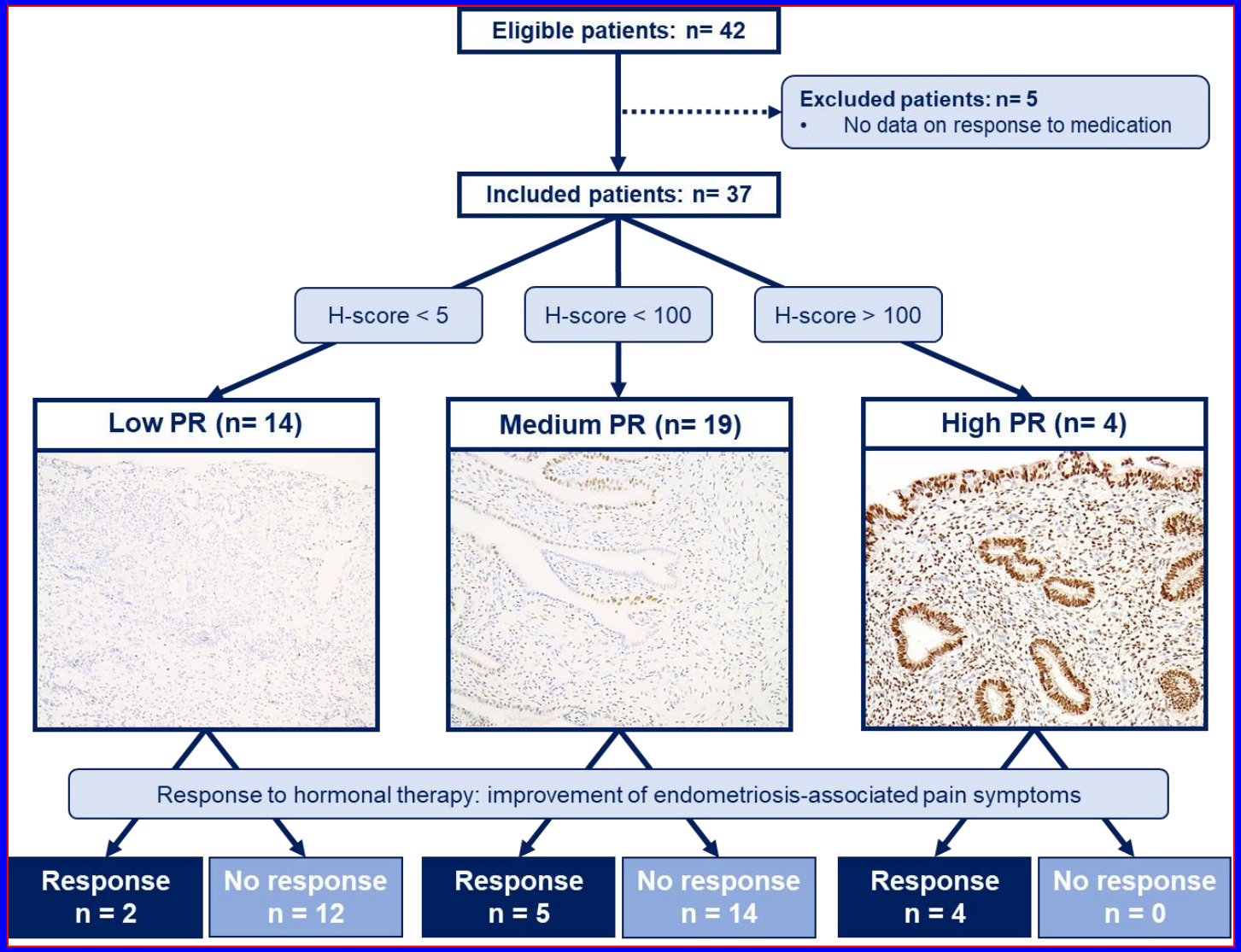
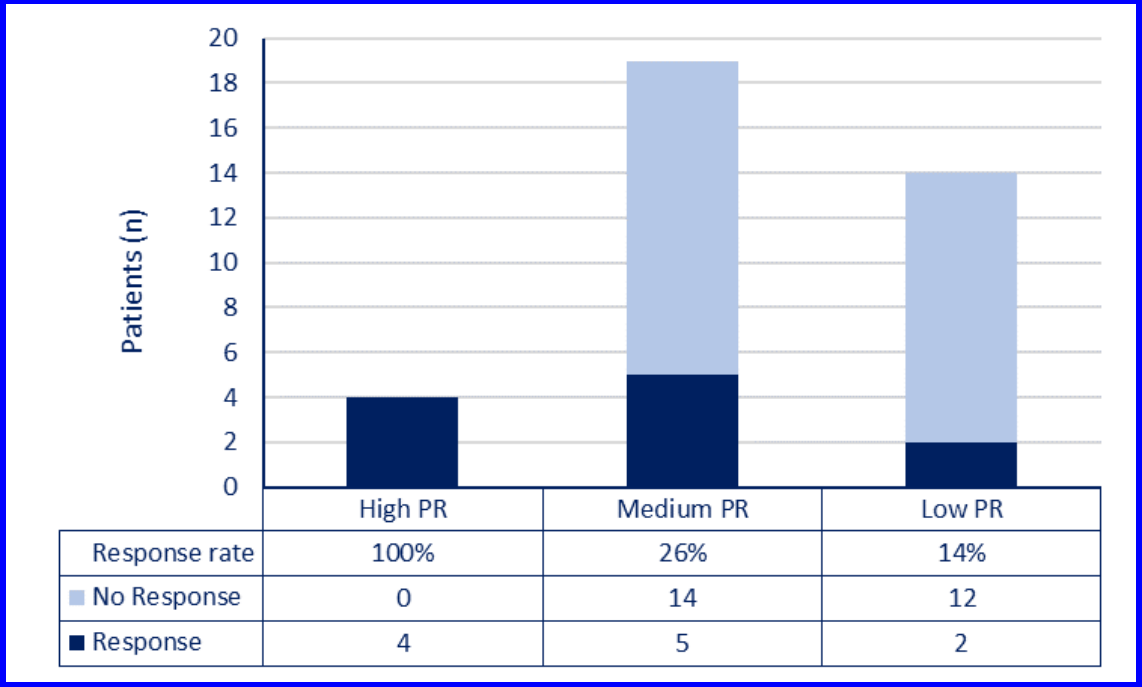
- Biomarkers for early detection/diagnosis
- Disease outcomes: prognostic biomarkers
- Treatment outcome: predictive biomarkers

Markers of Progestin Resistance



Flores V at al JCEM 2017



	High PR	Medium PR	Low PR
Response rate	100%	26%	14%
No Response	0	14	12
Response	4	5	2

- PR status was significantly associated (**p= 0.004**) with response to progestin therapy.
- All patients with high PR responded to progestin therapy.
- 86% of patients in the low PR group did not respond to progestin therapy.
- The medium PR group had a response rate of 26%

Changing the Timeline