

# MicroRNA expression in adolescents and young women with endometriosis

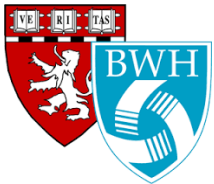


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**NEW ENGLAND FERTILITY SOCIETY  
MAY 6, 2017**



# Disclosure



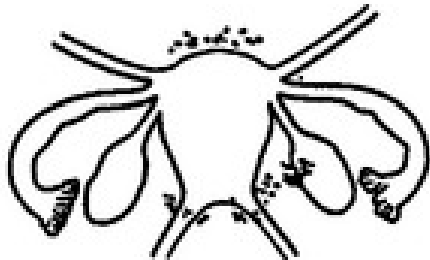
- **I have no financial relationship with a commercial entity producing health-care related products and/or services**

# Endometriosis

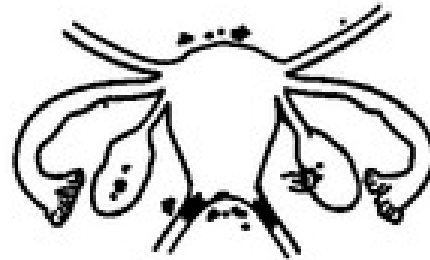


- Implantation of endometrial glands/stroma outside of the uterus, in up to 10% of women.
- Associated with pelvic pain, dyspareunia, dysmenorrhea, and bladder and bowel dysfunction.
- Infertility in 30-50%: Altered pelvic anatomy, inflammatory and immune function, oocyte quality.

STAGE I (MINIMAL)



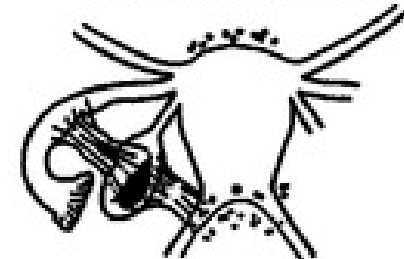
STAGE II (MILD)



STAGE III (MODERATE)



STAGE IV (SEVERE)



# Endometriosis



- Symptoms often start at menarche, but diagnosis is often delayed (on average 6.7 years)
- Early identification and intervention can slow disease progression and may improve fertility and functional outcomes.
- Currently, definitive diagnosis requires surgical visualization



# A Non-Invasive Test for Endometriosis

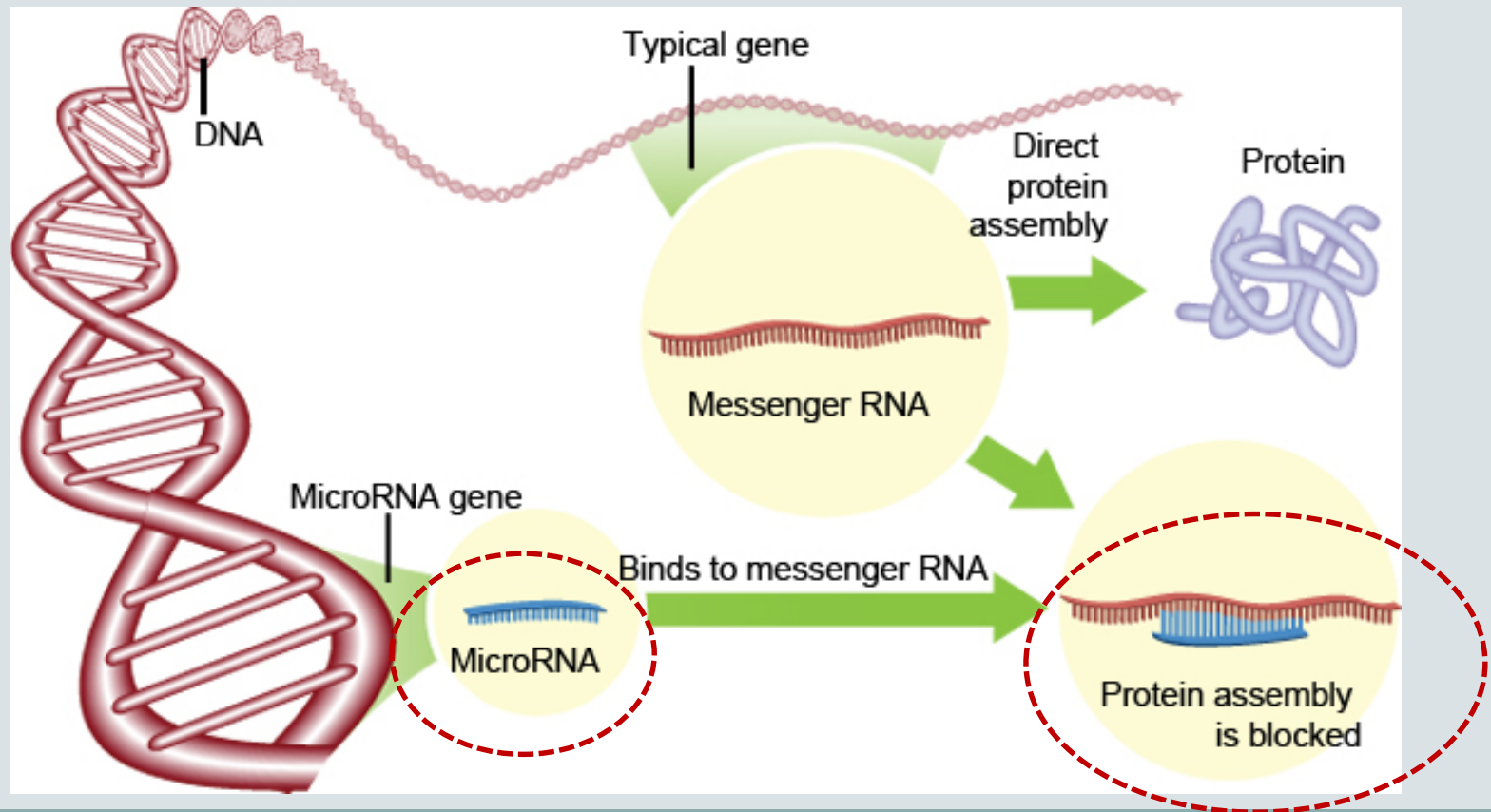


- Earlier diagnosis and referral to specialists
- Diagnosis for patients with geographic, insurance or other barriers to specialist referral
- Patients could potentially avoid unnecessary surgery
- Monitoring endometriosis activity and recurrence (avoid reoperation)
- Insights into the currently enigmatic pathogenesis of disease
- In the future: miRNA profile of endometriosis may allow for personalized precision medical treatment

# MiRNA



- Small noncoding RNA involved in epigenetic gene regulation largely through messenger RNA silencing.



# MiRNA Utility



- **MiRNA are identified in a wide variety of biologic samples, including tissues, serum, plasma, saliva and urine**
- **RNAase-resistant, therefore biologically stable.**
- **MiRNA profiles of endometriosis and ectopic pregnancies, among other gynecologic conditions, are starting to be elucidated**

# MiRNA and Endometriosis



- Expression of over 20 specific miRNA shown to be altered in adults with endometriosis.
  - miRNA 16, 17, 18a, 20a, 21, 22, 29c, 34c, 100, 125a, 126, 141, 145, 195, 199a, 200a, 200b, 200c, 202, 222, 424, 449
- Inverse correlations are observed between certain miRNA dysregulated in endometriosis and
  - VEGF-A (upregulating angiogenesis)
  - COX-2 (an enzyme involved in inflammation)
  - TGF-beta (regulating cell growth, proliferation, differentiation and apoptosis).



# Clinical application of miRNA



Discovery



mir-298 mir-23 mir-200  
mir-130 mir-18 mir-17 mir-505  
mir-20 mir-137 mir-92  
mir-495 mir-222  
mir-381 mir-148  
mir-19 m  
mir-21 let-7 mir-1  
mir-211 mir-7 mir-145  
mir-451 mir-15

Confirmation



Phase I

Clinical trial/use

- Though none are currently used in patient care, miRNA-based diagnostic tests for clinical use are in development.

# Need for research



- Existing studies of miRNA profiles in endometriosis:
  - Small
  - Span endometriosis stages I-IV
  - Include only adults
  - Inconsistent in biologic sample analyzed (serum, plasma, peritoneal fluid, eutopic or ectopic endometrium, endometriomas)
- These issues limit comparisons among studies, and to adolescents

# Specific Aims



- To test the hypothesis that certain miRNA are differentially expressed in adolescent patients with surgically-confirmed endometriosis, as compared to age-matched healthy controls.
- **Specific Aim 1: “Discovery”**—To perform quantitative real-time PCR using plasma from a representative subset of adolescent patients and age-matched controls.
- **Specific Aim 2:** To externally validate dysregulated miRNAs identified in Specific Aim 1, using the FirePlex™ Circulating microRNA Assay (Abcam®, Cambridge, MA), in a replication set of case samples and age-matched controls.

# Study samples



- **Case samples:** Females 13 - 26 years with surgically-confirmed endometriosis, enrolled in **The Women's Health Study: From Adolescence to Adulthood**, a longitudinal cohort study of endometriosis across the lifespan.
- **Controls:** Healthy volunteers, age 13-26, without signs or symptoms of endometriosis



**The Women's  
Health Study**  
*From Adolescence to Adulthood*

# Study samples



- Plasma collected and processed according to World Endometriosis Research Foundation (WERF) Endometriosis Phenome and Biobanking Harmonization Project (EPHect) guidelines

**World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonization Project: III. Fluid biospecimen collection, processing, and storage in endometriosis research**

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**Fertility and Sterility.**

- Blood centrifuged 2,500 x g for 10 minutes at 4°C; the plasma was aspirated, aliquoted into cryovials, then frozen to -80°C

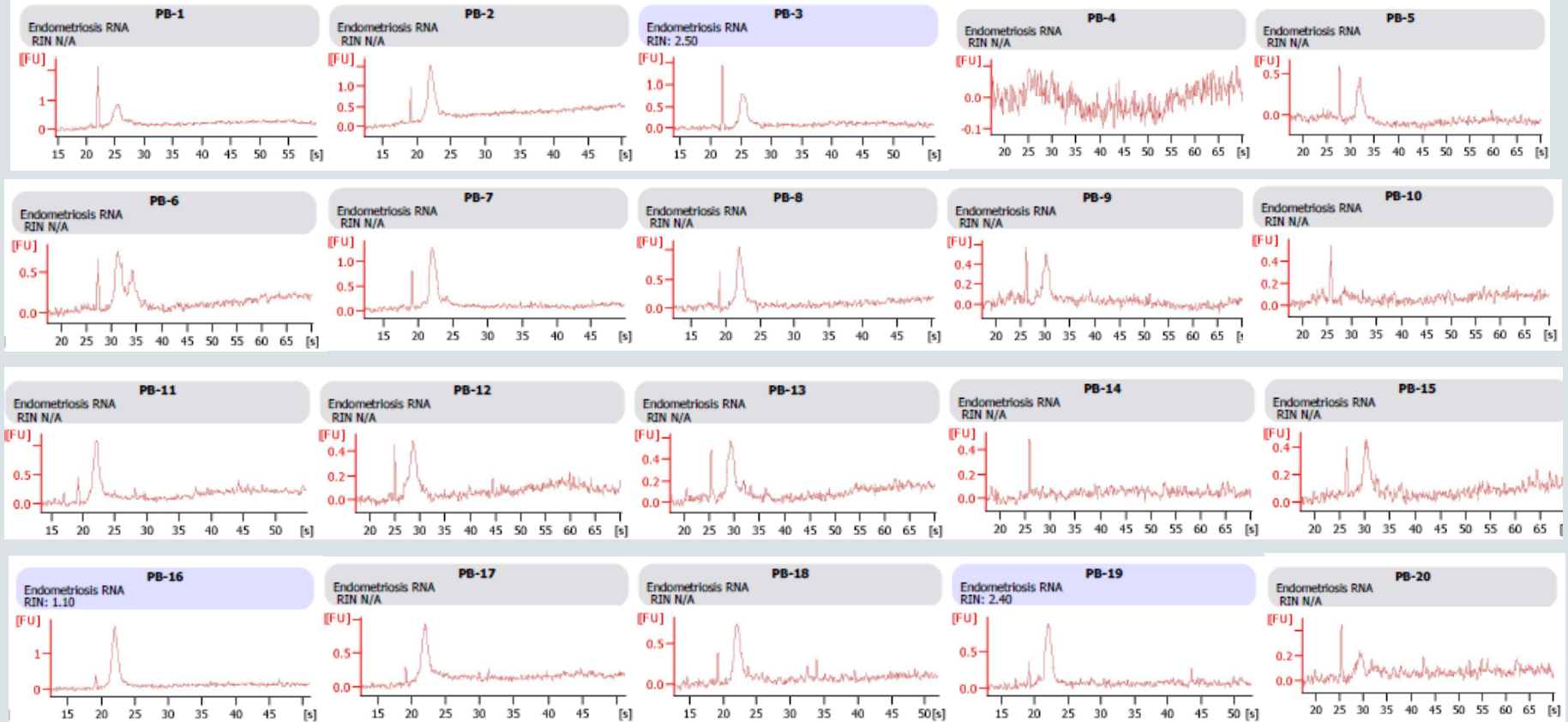


# Discovery phase



- Matched 10 pairs of cases and controls
- 5 pairs using hormonal medications at the time of sample collection, 5 without hormone use for at least 30 days prior to collection
- Samples collected before any surgery performed, or  $\geq 6$  months postoperatively
- Cases and controls age-matched within 2 years
- For discovery phase, excluded patients with known inflammatory or autoimmune disease

# MiRNA extraction and quantification

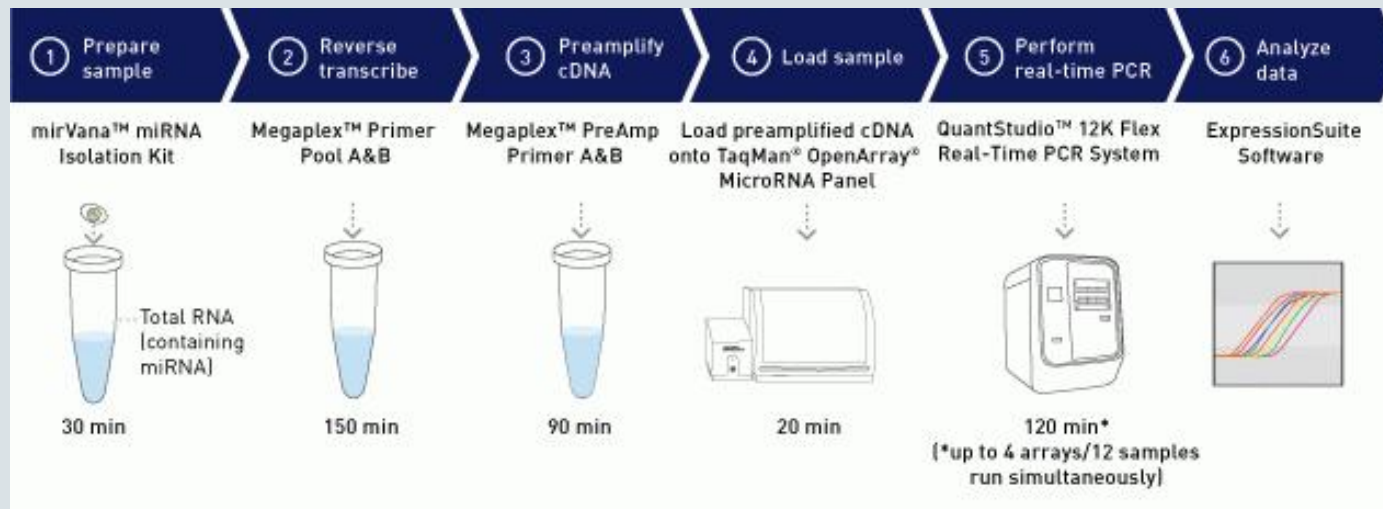




# Quantitative real-time PCR



- Applied Biosystems TaqMan<sup>®</sup> Array MiRNA 384 well cards x 2
- Analyze 760+ miRNA in each sample
- Relative miRNA expression between cases and controls calculated using the  $2^{-\Delta\Delta C_t}$  method.



# Preliminary results

Prostaglandin F2  
receptor inhibitor



colony  
stimulating  
factor 1  
(macrophage)

GABA-B-R

## • Upregulated:

- miR-1180
- miR-1183
- miR-122
- miR-1291
- miR-135b
- miR-144
- miR-15b
- miR-183
- miR-18a
- miR-202
- miR-204
- miR-205
- miR-208
- miR-218
- miR-25

- miR-30a-3p
- miR-381
- miR-429
- miR-450b-5p
- miR-483-3p
- miR-489
- miR-501-3p
- miR-502
- miR-511
- miR-519b-3p
- miR-624
- miR-708
- miR-939
- miR-95

## • Downregulated:

- miR-1276
- miR-1298
- miR-148a
- miR-181c
- miR-331-5p
- miR-487a
- miR-520D-3p
- miR-551b
- miR-571
- miR-597
- miR-654
- miR-661
- miR-770-5p
- miR-885-5p

TGF-BR1

TNF-R

MMP-24

estrogen-  
related  
receptor-α

TNF

IGF2

prostaglandin G/H synthase and cyclooxygenase

TGF-BR1

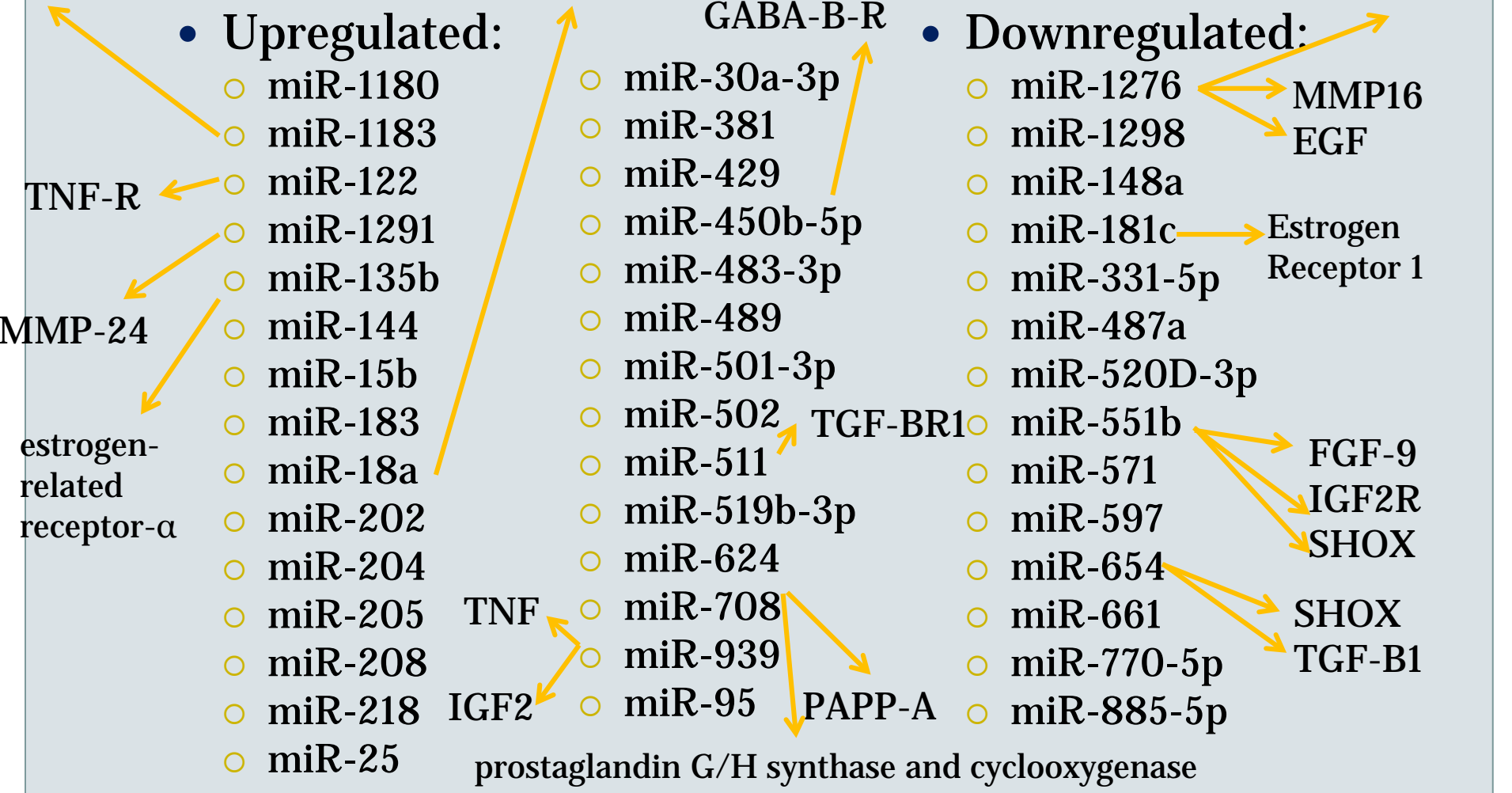
PAPP-A

MMP16  
EGF

Estrogen  
Receptor 1

FGF-9  
IGF2R  
SHOX

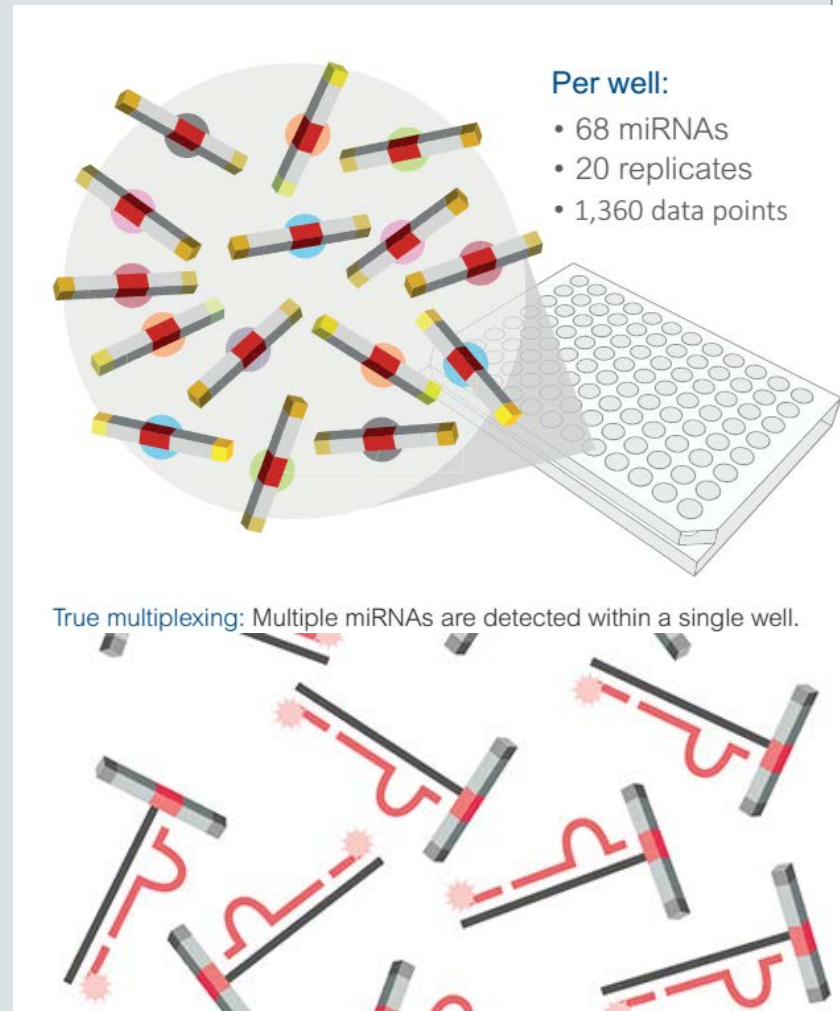
SHOX  
TGF-B1



# Next Step: Confirmation



- **FirePlex: Multiplex circulating miRNA assay**
- **68 miRNA per well, 96 wells**
- **Hydrogel technology—each well contains particles with code to identify individual miRNA**
- **Readout is performed on flow cytometer**



# Thank you

- New England Fertility Society
- Ferring
- Stacey Missmer, ScD
- Allison Vitonis, ScM
- Sigitas Verselis, PhD



*Boston Center for Endometriosis*



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