SERUM MICRORNA PROFILING FOR THE IDENTIFICATION OF PREDICTIVE MOLECULAR MARKERS OF THE RESPONSE TO CONTROLLED OVARIAN STIMULATION

Edson Borges Jr; Maria Gabriela Ferreira Mulato; Amanda Souza Setti; Assumpto Iaconelli Jr. ; Murilo Vieira Geraldo; Daniela Paes de Almeida Ferreira Braga

FERTILITY MEDICAL GROUP

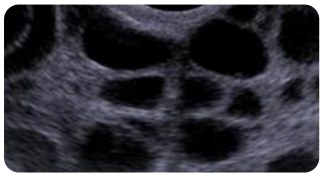
Associação Instituto Sapientiae

UNICAMP

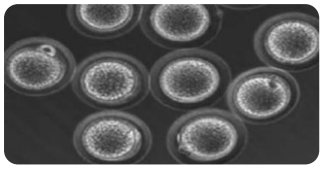
INTRODUCTON

✓ Controlled Ovarian Stimulation

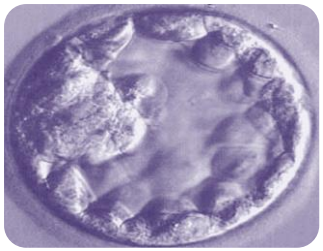
Essential step for in vitro fertilization



Development of multiple follicles



Recovery of multiple oocytes



Selection of the best embryo for transfer

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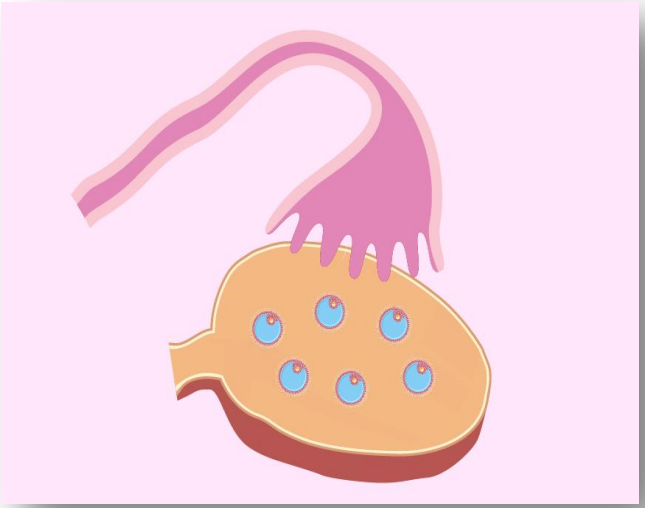
INTRODUCTION

✓ Controlled Ovarian Stimulation



Proper response to COS → 5 to14 oocytes

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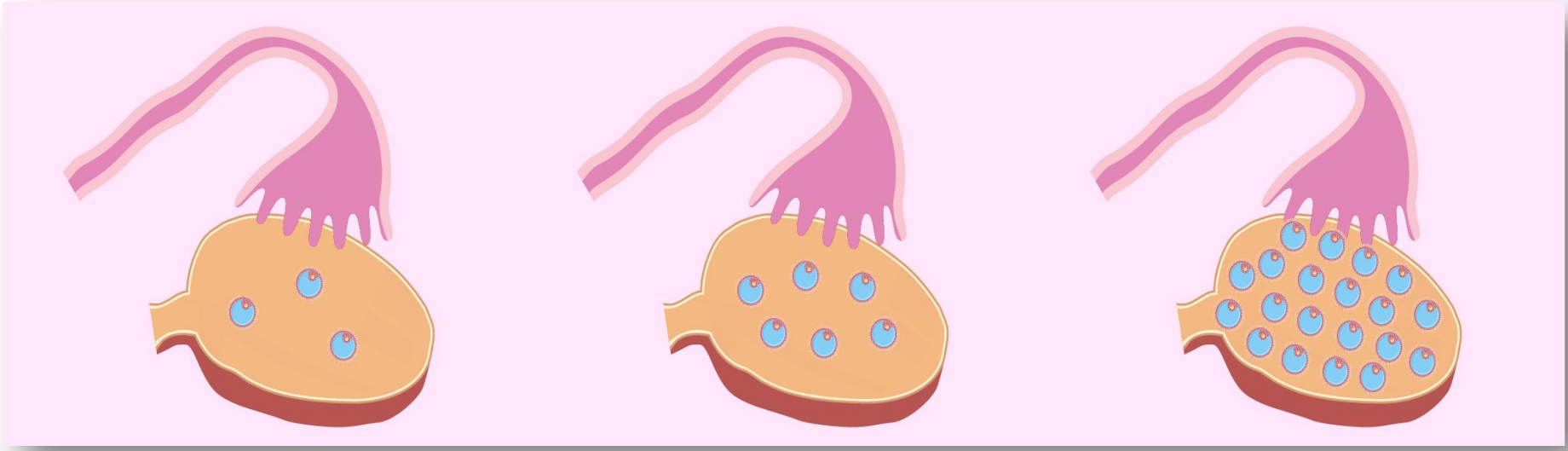
Cai et al. 2013 Briggs et al. 2014

INTRODUCTION

✓ Controlled Ovarian Stimulation



Poor response → Normo response → Hyper response

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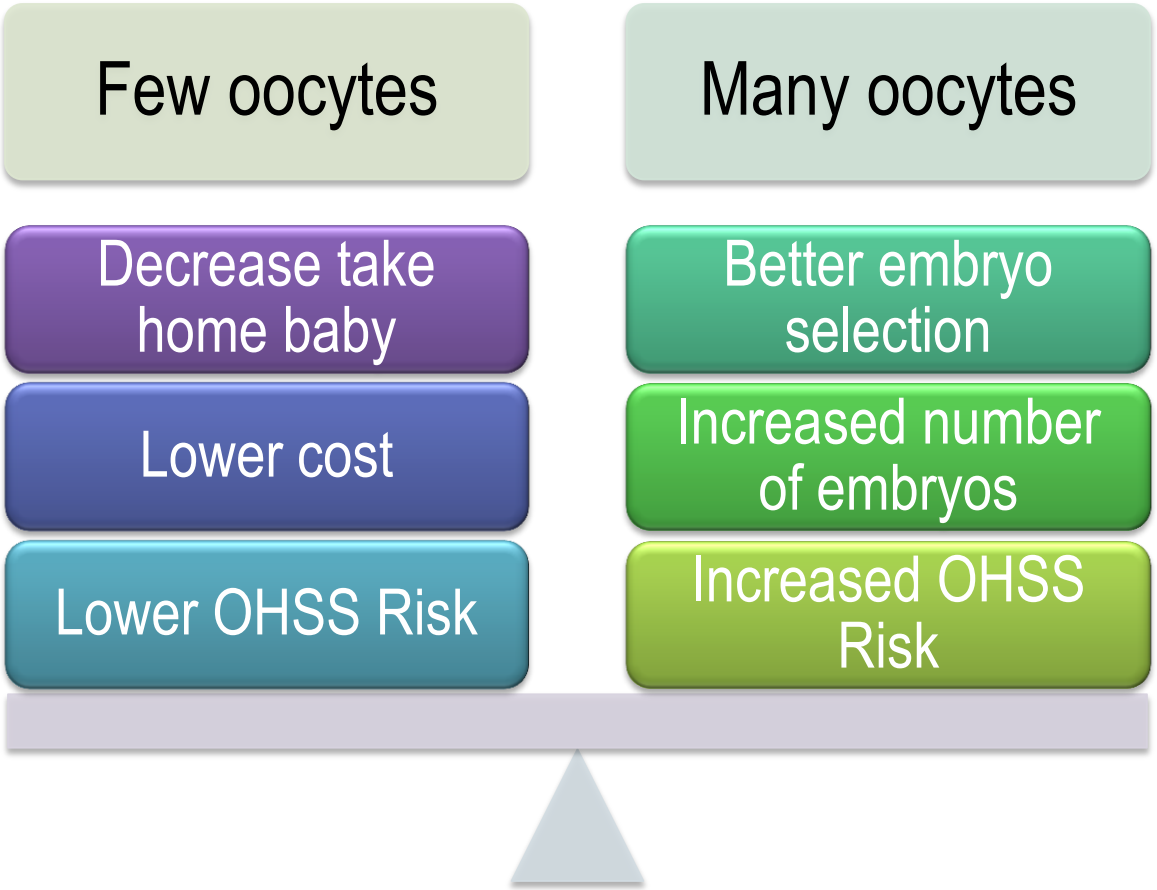
INTRODUCTION

✓ Cancellation of IVF cycles risk



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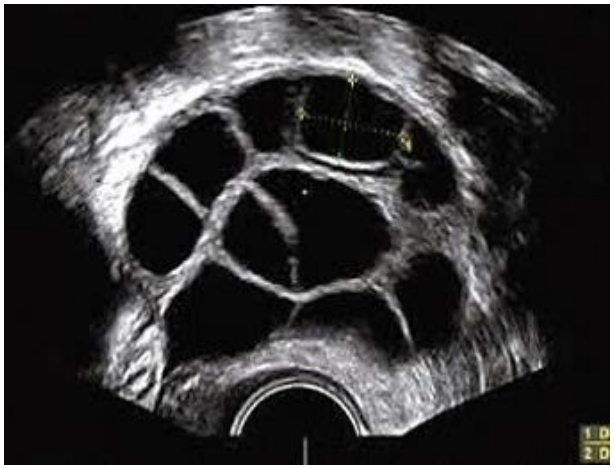
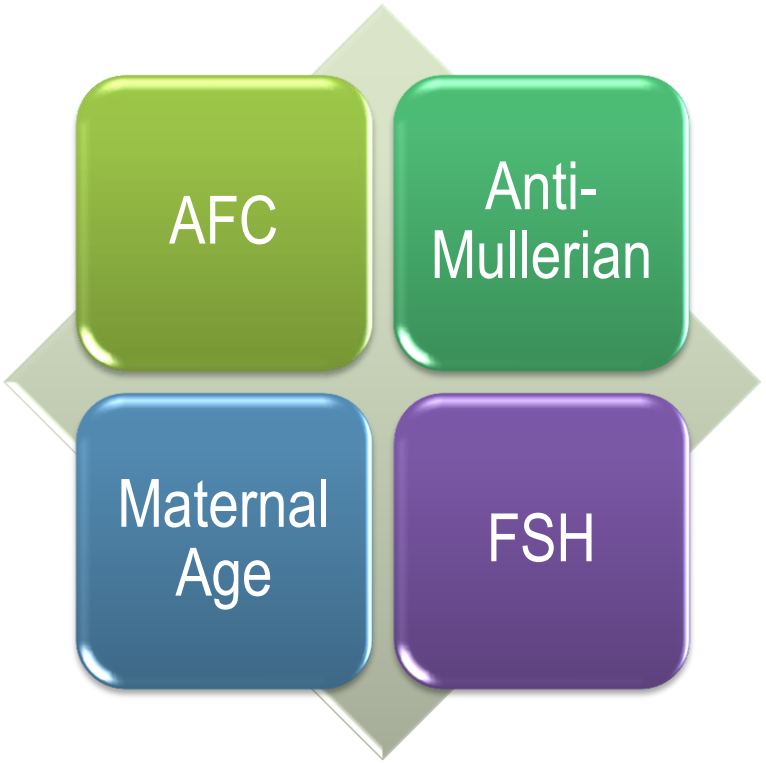
Kumbak et al. 2009, Sunkara et al 2011

INTRODUÇÃO

- ✓ It is nearly impossible to accurately predict the ovarian response and tailor an individualised stimulation protocol



+



The development of non-invasive techniques, capable of predicting the response to COS, would allow for individualised treatment and increased success rates.

INTRODUCTION

- **MicroRNAs**

Endogenous

Single-strand RNA molecules

of 20-24 nucleotides

Non-coding RNA molecule

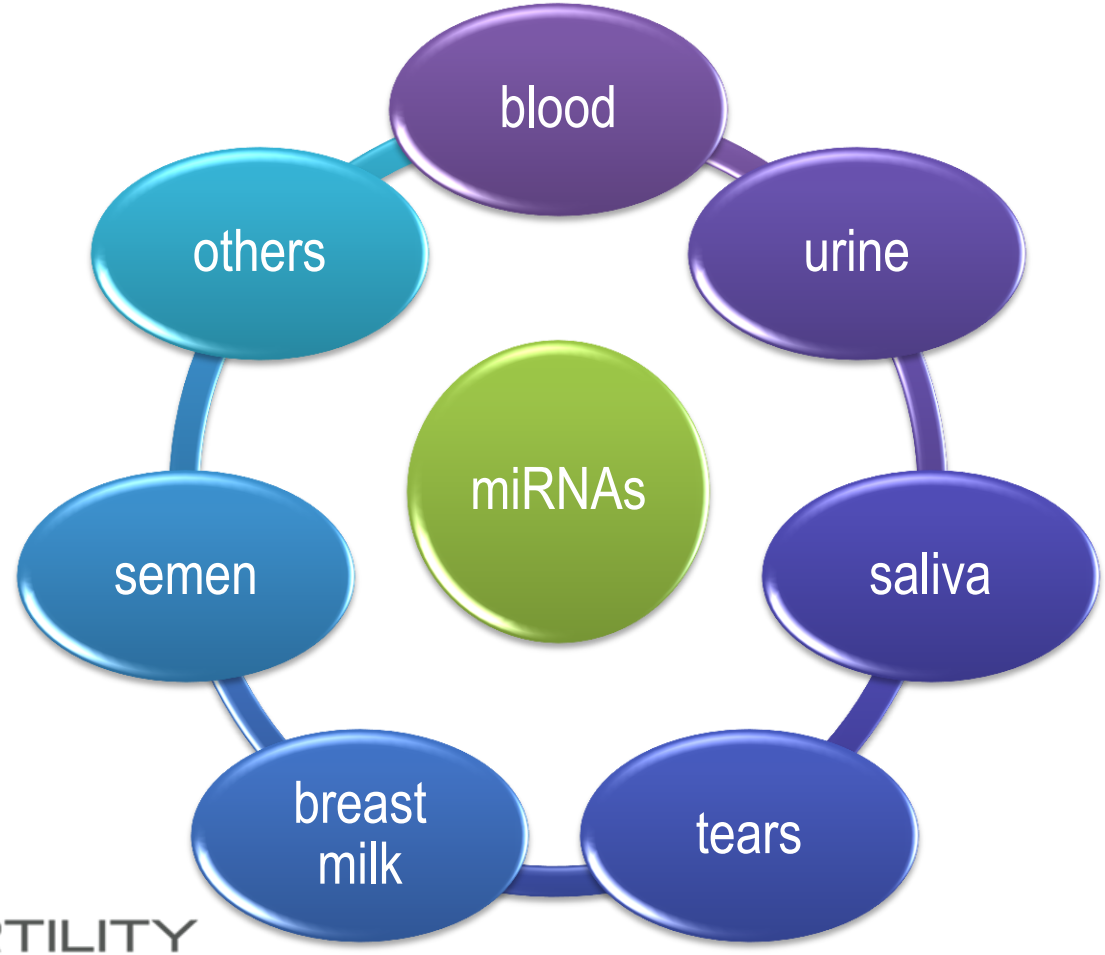
Post-transcriptionally regulate gene expression in eukaryote

FERTILITY

Asirvatham et al. 2009, McCallie et al. 2010, Mouillet et al. 2015, Thouas et al. 2015

INTRODUCTION

- **MicroRNAs:** actively secreted into the bloodstream and regulate gene expression in distant organs



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Thomou et al. 2017, Wang et al. 2010, Weber et al. 2010

OBJECTIVE

Identify potential miRNAs biomarkers of ovarian response to controlled ovarian stimulation

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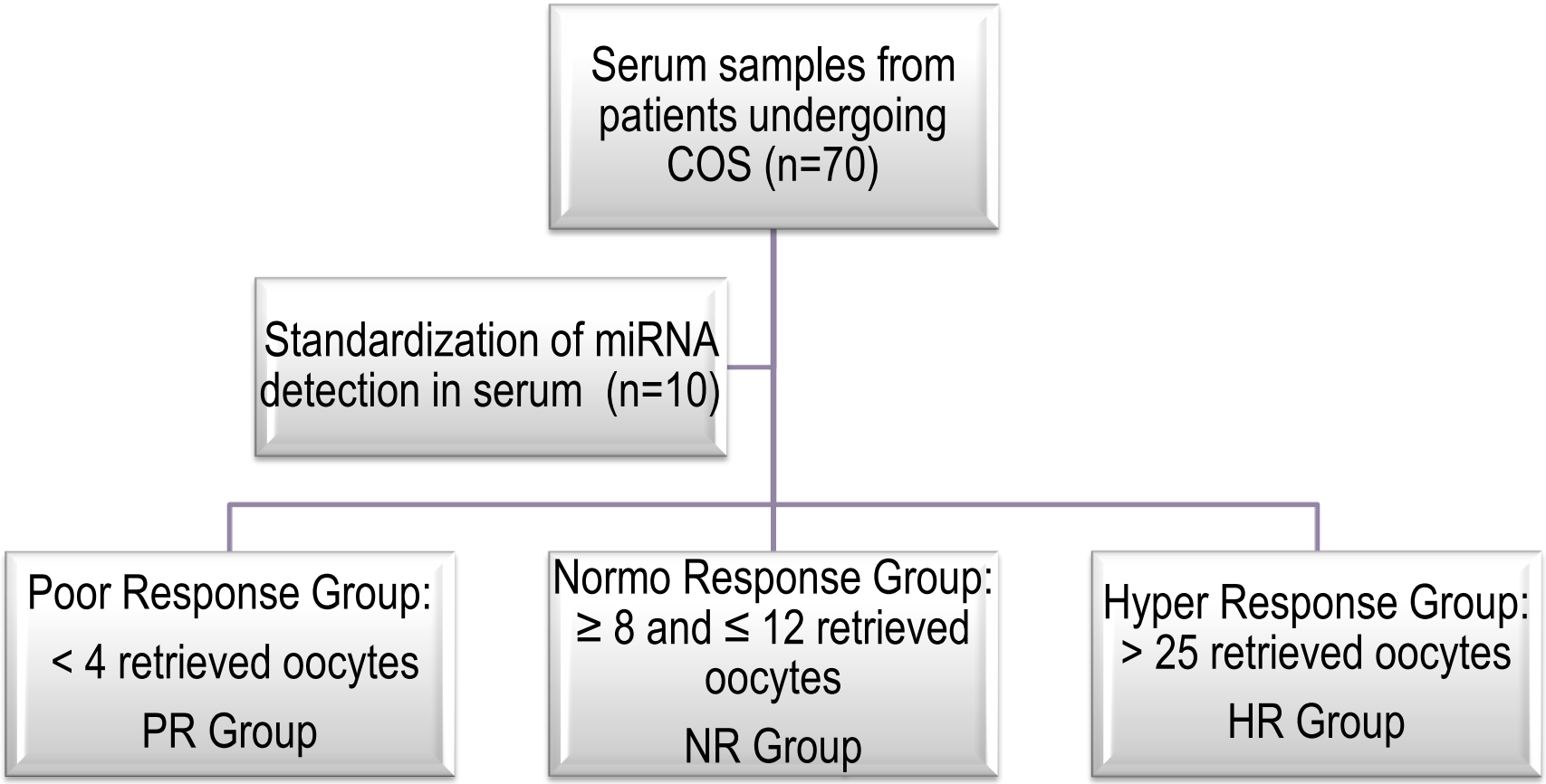
MATERIAL AND METHODS

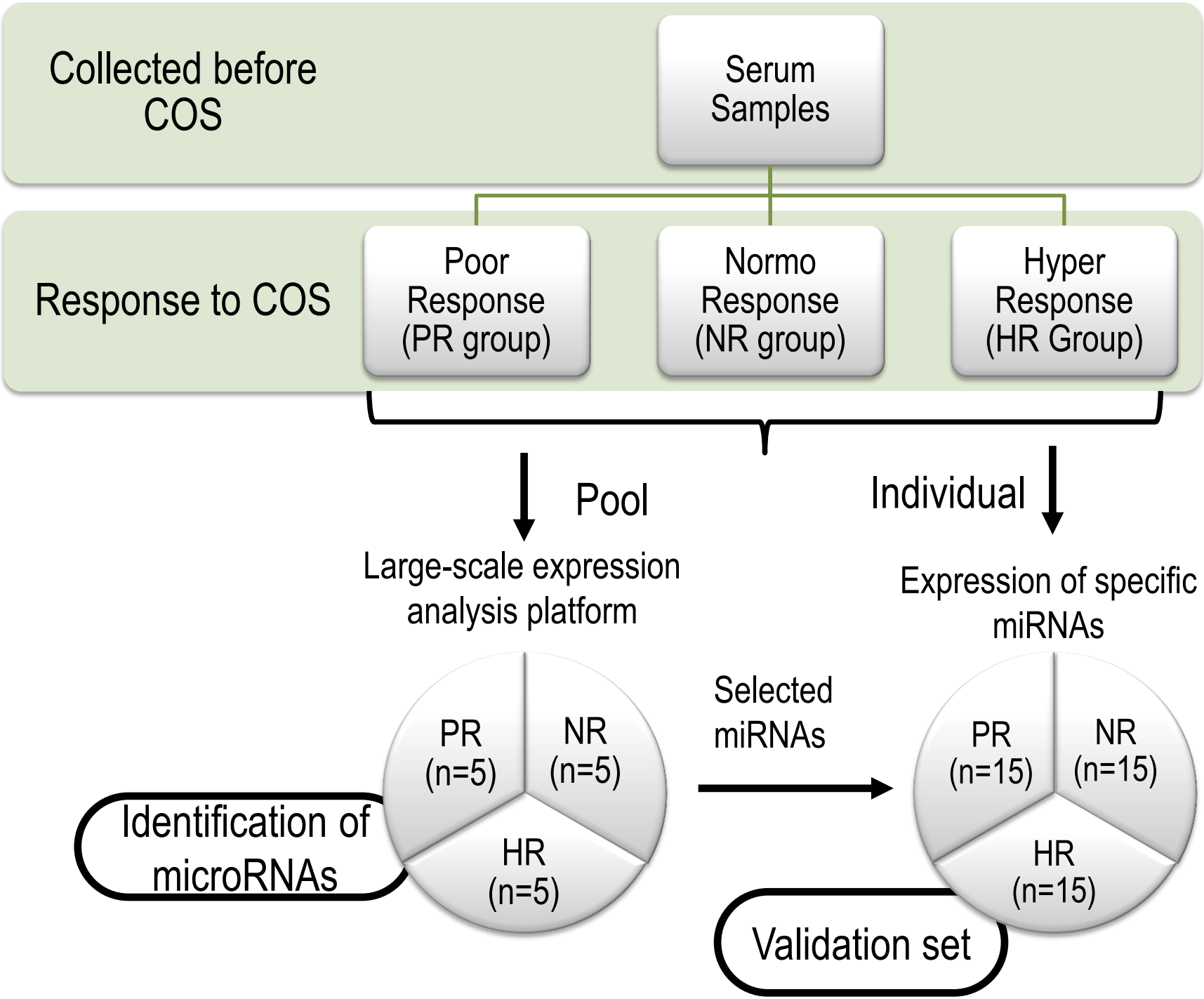


MATERIAL AND METHODS

Two experimental sets (60 samples)

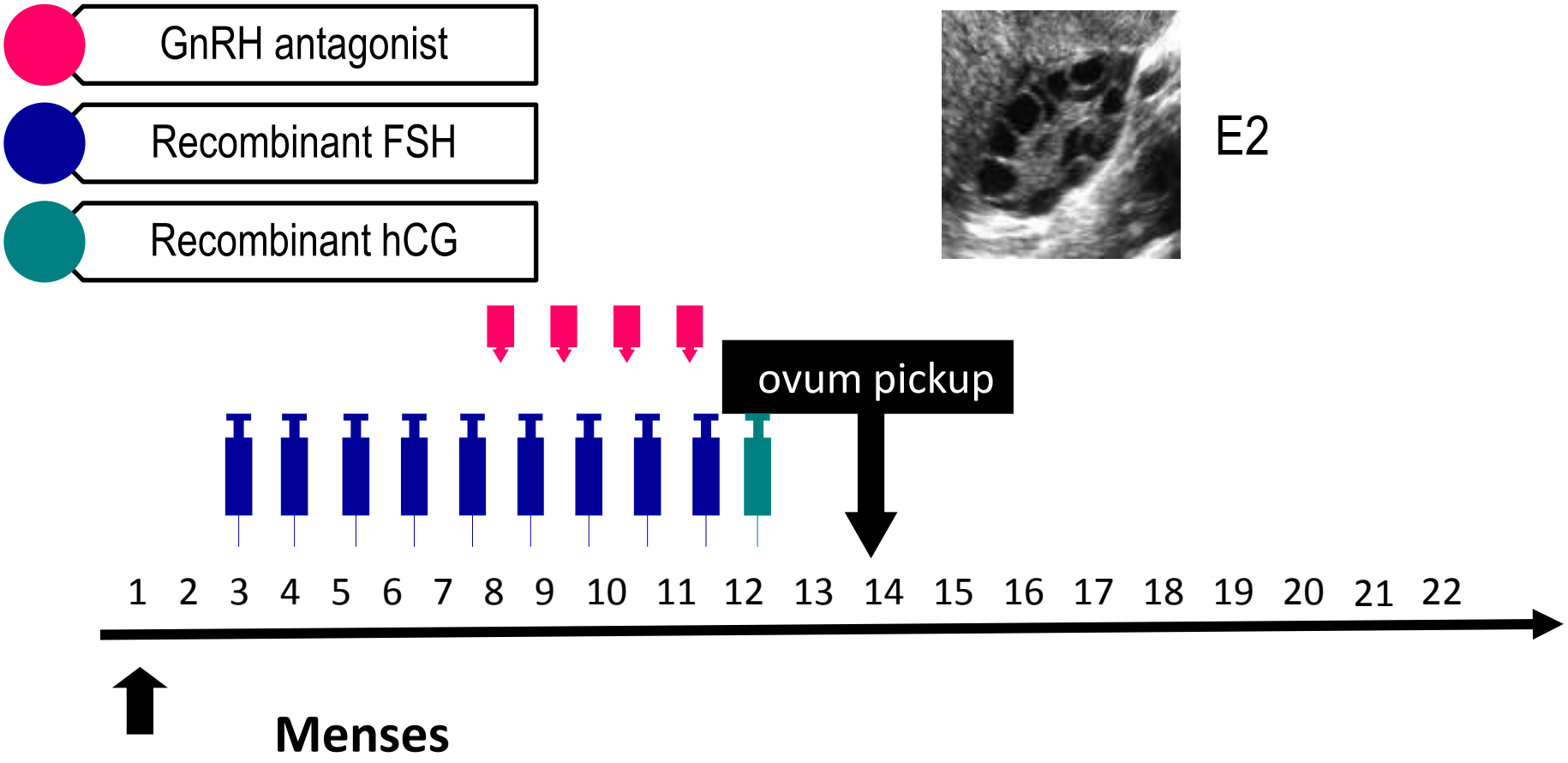
Identification of aberrantly expressed miRNAs (n=15)

Validation set (n=45)


Collected before COS
Serum Samples
Response to COS
Poor Response (PR group)
Normo Response (NR group)
Hyper Response (HR Group)
Pool
Individual
Large-scale expression analysis platform
Expression of specific miRNAs
PR (n=5)
NR (n=5)
HR (n=5)
Identification of microRNAs
Selected miRNAs
PR (n=15)
NR (n=15)
HR (n=15)
Validation set

MATERIAL AND METHODS

✓ Controlled Ovarian Stimulation



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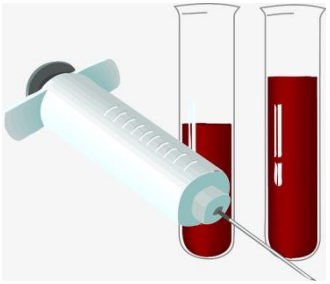
MATERIAL AND METHODS

Serum samples obtained through venipuncture, prior to beginning of COS



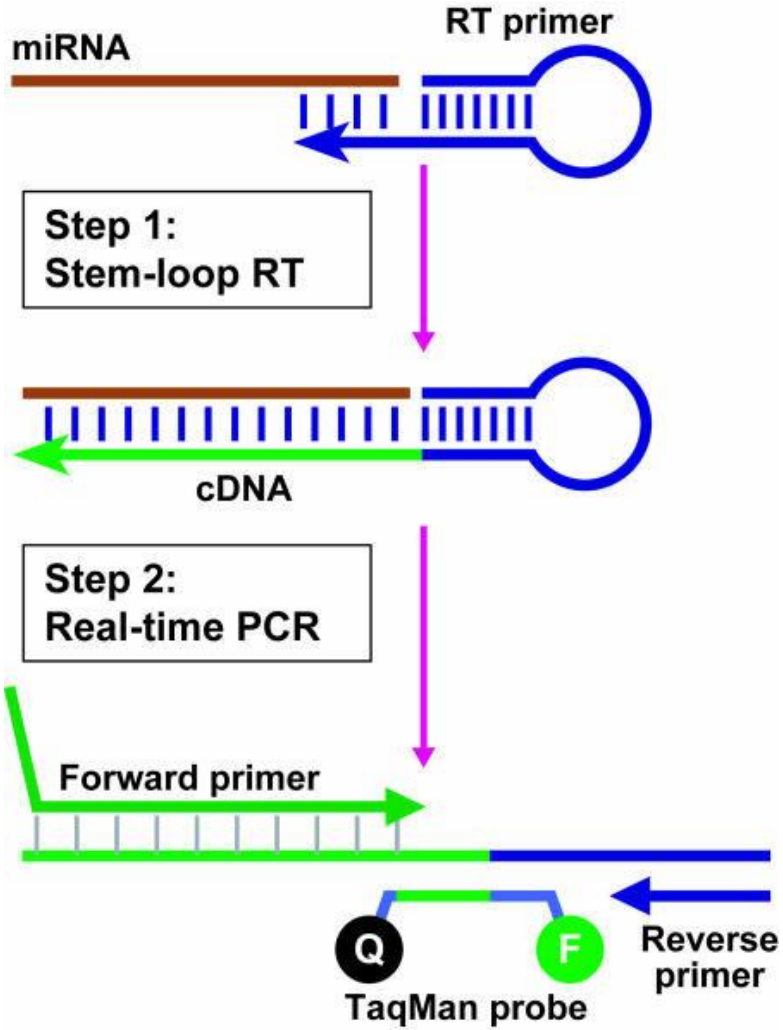
Small RNAs was extracted from 200µl of serum samples using a commercial kit

Identification and quantification of aberrantly expressed miRNAs performed by using a large-scale quantification PCR-Array

MATERIAL AND METHODS

According with this analysis, four miRNAs were selected to be tested in the validation set

The validation was performed by using the miRNA Assays quantification kit



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RESULTS

Patient's and cycle's characteristics for the Poor response, Normo response and Hyper response Groups

	PR (n=5)	NR (n=5)	HR (n=5)	p
Age (years)	33.88 ± 1.87	32.40 ± 2.75	31.30 ± 2.11	0.065
BMI (kg/m^2)	25.04 ± 4.27	22.44 ± 2.50	25.50 ± 4.19	0.264
FSH dose (IU)	2383.33 ± 668.48	2550.00 ± 469.87	2495.00 ± 584.38	0.825
Estradiol level (pg/ml)	913.00 ± 415.80^a	1818.00 ± 1073.19^b	3901.00 ± 770.74^c	0.003
Aspirated follicles (n)	3.50 ± 0.85^a	11.20 ± 1.03^b	50.40 ± 22.24^c	<0.001
Retrieved oocytes (n)	2.80 ± 0.92^a	9.70 ± 1.49^b	36.50 ± 4.45^c	<0.001
Oocyte retrieval rate (%)	82.50 ± 23.71	86.82 ± 11.84	81.03 ± 24.98	0.816
Mature oocytes (n)	2.40 ± 0.84^a	7.30 ± 1.56^b	23.80 ± 5.09^c	<0.001

RESULTS

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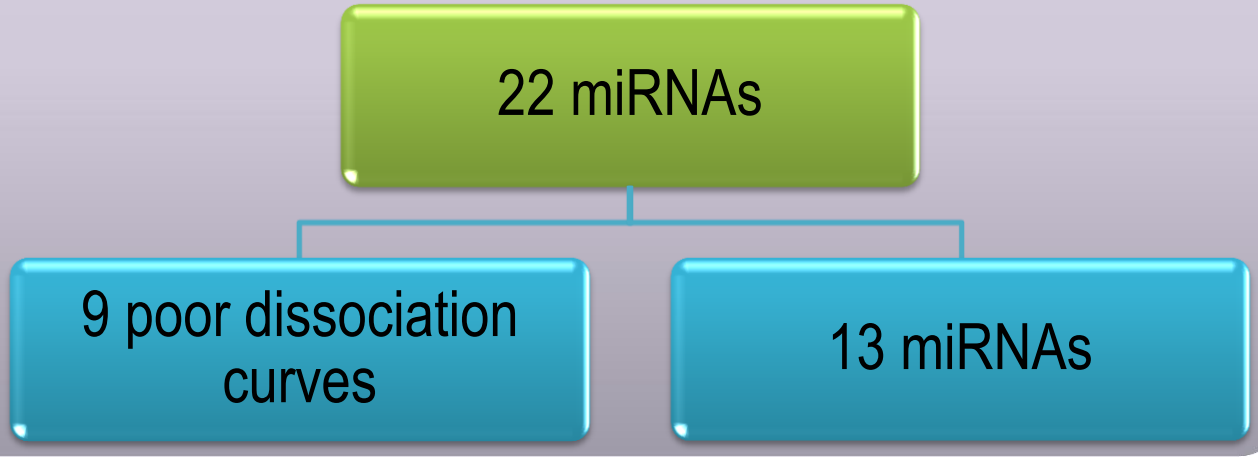
RESULTS

Fold Change twofold increased expression in the PR or HR groups vs NR group



PR

miR-150-5p
miR-223-3p
let-7d-3p
miR-891a-5p
miR-99a-3p
miR-200c-5p

HR

miR-99a-5p
miR-181d-5p
miR-221-3p
miR-92a-1-5p

RESULTS

Quality of the amplification

Detection pattern in the experimental groups

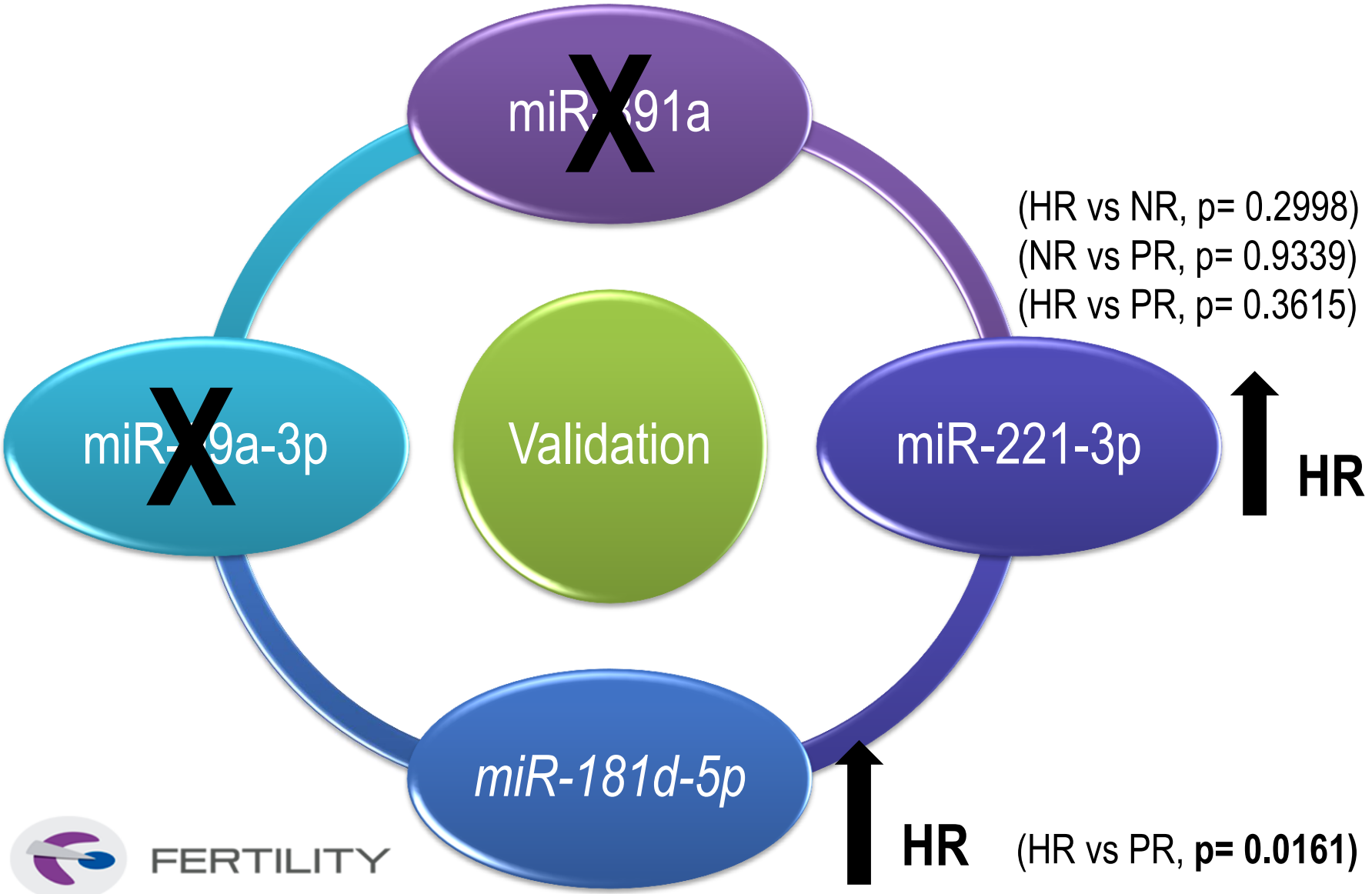
Literature data

PR group
(miR-891a-5p and miR-99a-3p)

HR group
(miR-181d-5p and miR- 221-3p)

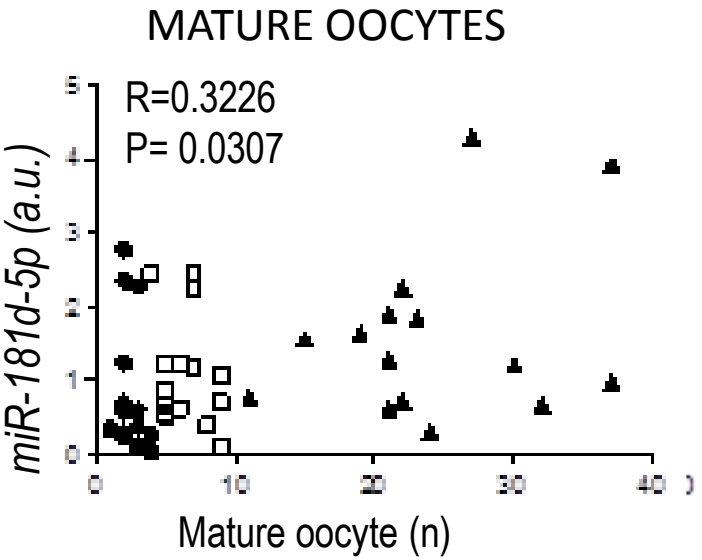
SELECTED FOR THE VALIDATION

RESULTS



FERTILITY

RESULTS


MATURE OOCYTES
R=0.3226
P= 0.0307
miR-181d-5p (a.u.)
0
1
2
3
4
5
0
10
20
30
40
Mature oocyte (n)

CONCLUSION

miRNA biomarker: potential diagnostic toll for the management of the treatment of infertility

The quantification of *miR-181d-5p* prior to the COS may discriminate patients who will hyper or poor respond to the COS

Allowing the individualization of the treatment, increasing treatment success while decreasing the risks, patients' physical, emotional and economic burden

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DISCUSSÃO

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DISCUSSÃO

Primeiro estudo que correlaciona metabólitos sanguíneos e resposta ovariana

REPRODUCTION
RESEARCH

Prediction of embryo implantation potential by mass spectrometry fingerprinting of the culture medium

Sylvia Sanches Cortezzi[1], Elaine Cristina Cabral[2], Marcello Garcia Trevisan[3,4], Christina Ramires Ferreira[2], Amanda Souza Setti[1,5], Daniela Paes de Almeida Ferreira Braga[1,5], Rita de Cássia Sávio Figueira[5], Assumpto Iaconelli Jr[1,5], Marcos Nogueira Eberlin[2] and Edson Borges Jr[1,5]

JBRA Assisted Reproduction 2016;**20**(4):227-231
doi: 10.5935/1518-0557.20160044

Original Article

P-658 Wednesday, November 1, 2017

UTERINE FLUID LIPIDOMICS AS AN ENDOMETRIAL RECEPTIVITY PREDICTIVE TOOL. D. P. Braga,[a,b,c] D. A. Montanni,[c] A. S. Setti,[a,b] G. Pilli,[d] A. Godoy,[d] M. N. Eberlin,[d] A. Iaconelli, Jr.,[a,b] E. Borges, Jr.,[a,b] E. G. Lo Turco.[c] [a]Fertility Medical Group, Sao Paulo, Brazil; [b]Instituto Sapientiae - Centro de Estudos e Pesquisa em Reprodução Assistida, Sao Paulo, Brazil; [c]Disciplina de Urologia, Departamento de Cirurgia – UNIFESP, Sao Paulo, Brazil; [d]Laboratório ThoMSon de Espectrometria de Massas – Instituto de Química - UNICAMP, Campinas, Brazil.

):119-124

Non-invasive prediction of blastocyst implantation, ongoing pregnancy and live birth, by mass spectrometry lipid fingerprinting

Edson Borges Jr.[1,2], Daniela P.A.F. Braga[1,2,3], Amanda Souza Setti[1,2], Daniela A. Montanni[3], Elaine Cristina Cabral[4], Marcos N. Eberlin[4], Edson G. Lo Turco[3], Assumpto Iaconelli Jr[1]

Original Article

FERTILITY

Non-Invasive Prediction of Blastocyst Formation by Day Three Embryo Culture Medium Mass Spectrometry Lipid Fingerprinting

Daniela Paes de Almeida Ferreira Braga[1,2,3], Amanda Souza Setti[2,3], Elaine Cristina Cabral[4], Marcos Eberlin[5], Edson Guimarães Loturco[1], Edson Borges Jr[2,3]

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FERTILITY

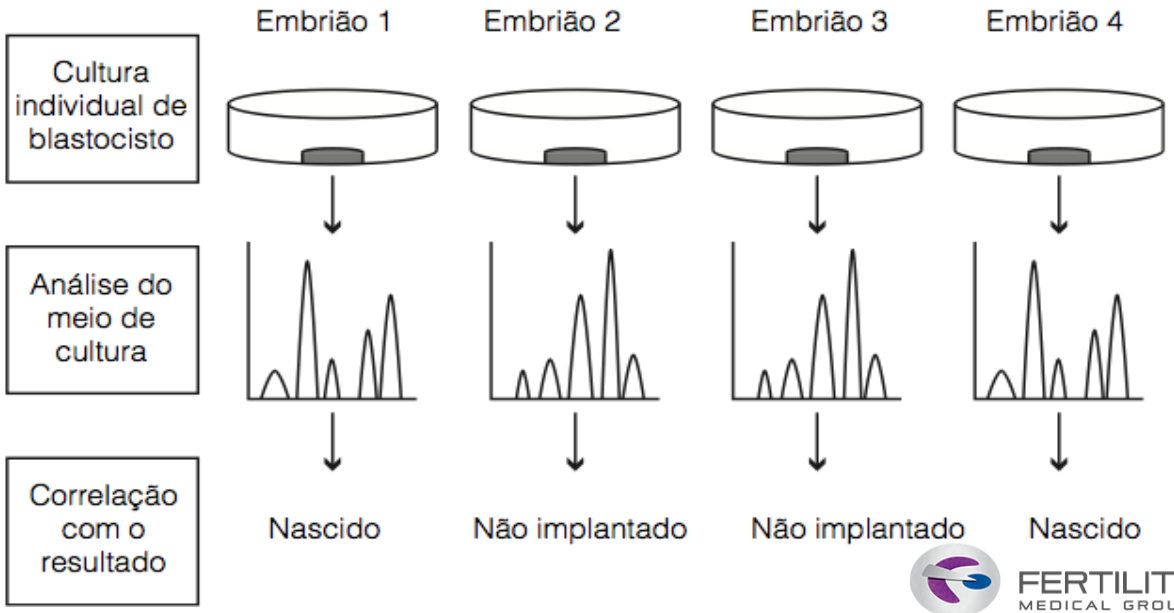
INTRODUÇÃO

Metabolômica

REPRODUCTION
RESEARCH

Prediction of embryo implantation potential by mass spectrometry fingerprinting of the culture medium

Sylvia Sanches Cortezzi[1], Elaine Cristina Cabral[2], Marcello Garcia Trevisan[3,4], Christina Ramires Ferreira[2], Amanda Souza Setti[1,5], Daniela Paes de Almeida Ferreira Braga[1,5], Rita de Cássia Sávio Figueira[5], Assumpto Iaconelli Jr[1,5], Marcos Nogueira Eberlin[2] and Edson Borges Jr[1,5]



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INTRODUÇÃO

Metabolômica

REPRODUCTION
RESEARCH

Metabolic profiling of human follicular fluid identifies potential biomarkers of oocyte developmental competence

A O'Gorman[1,2], M Wallace[1,2], E Cottell[3], M J Gibney[2], F M McAuliffe[4], M Wingfield[3] and L Brennan[1,2]

Human Reproduction, Vol.32, No.11 pp. 2269–2278, 2017
Advanced Access publication on October 13, 2017 doi:10.1093/humrep/dex303

human reproduction ORIGINAL ARTICLE *Reproductive biology*

Targeted metabolomics reveals reduced levels of polyunsaturated choline plasmalogens and a smaller dimethylarginine/arginine ratio in the follicular fluid of patients with a diminished ovarian reserve

J.M. Chao de la Barca[1,2], T. Boueilh[3], G. Simard[1,4], L. Boucret[2,3], V. Ferré-L'Hotellier[3], L. Tessier[1], C. Gadras[1], P.E. Bouet[5], P. Descamps[5], V. Procaccio[1,2], P. Reynier[1,2], and P. May-Panloup[2,3,*]

Molecular BioSystems

Metabolomic profiling and biochemical evaluation in follicular fluid of endometriosis patients

Santonastaso Marianna[1#], Pucciarelli Alessia[2#], Costantini Susan[3], Caprio Francesca[4], Sorice Angela[3], Capone Francesca[3], Natella Antonella[4], Iardino Patrizia[5], Colacurci Nicola[1*], Chiosi Emilio[2*]

www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol. 8, (No. 46), pp: 80472-80480

Research Paper

Follicular metabolic changes and effects on oocyte quality in polycystic ovary syndrome patients

Yan Zhang[2,*], Lingyan Liu[1,*], Tai-Lang Yin[4], Jing Yang[4] and Cheng-Liang Xiong[2,3]

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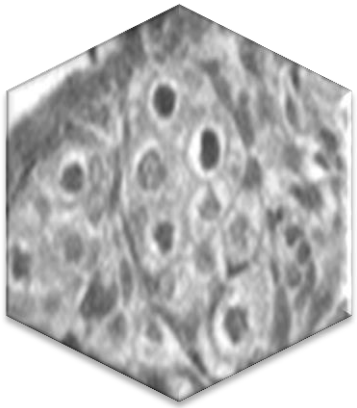
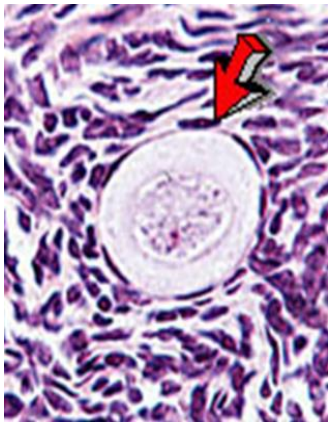
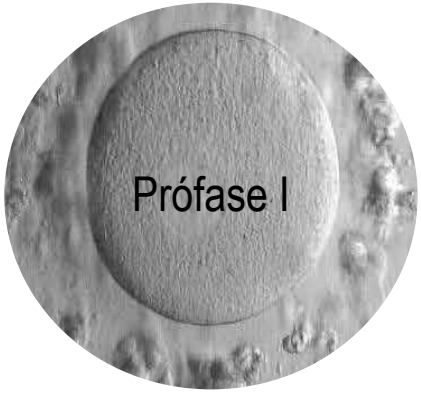
INTRODUÇÃO



Pool de folículos primordiais





Destino de cada folículo depende de fatores autócrinos e parácrinos

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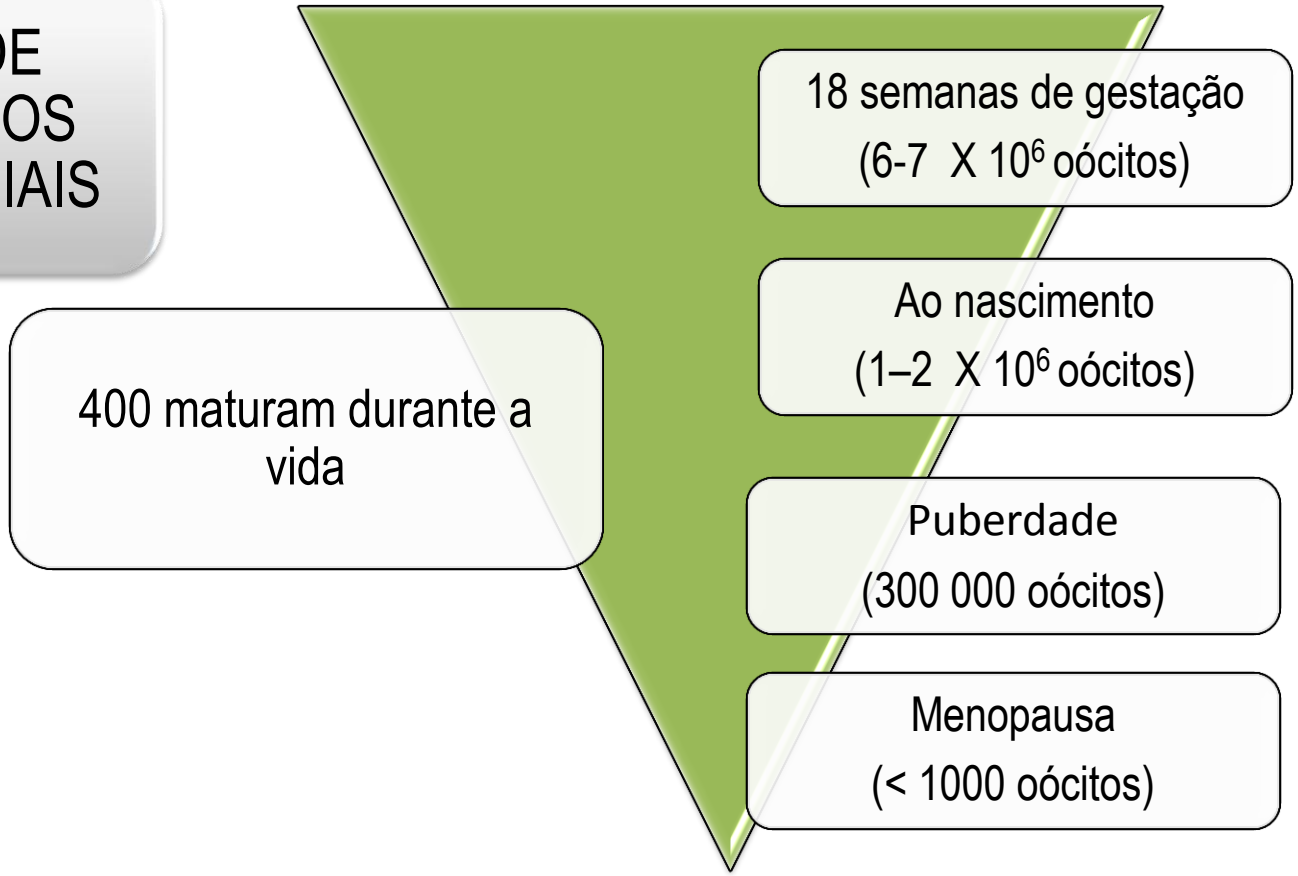
INTRODUÇÃO

POOL DE FOLÍCULOS PRIMORDIAIS



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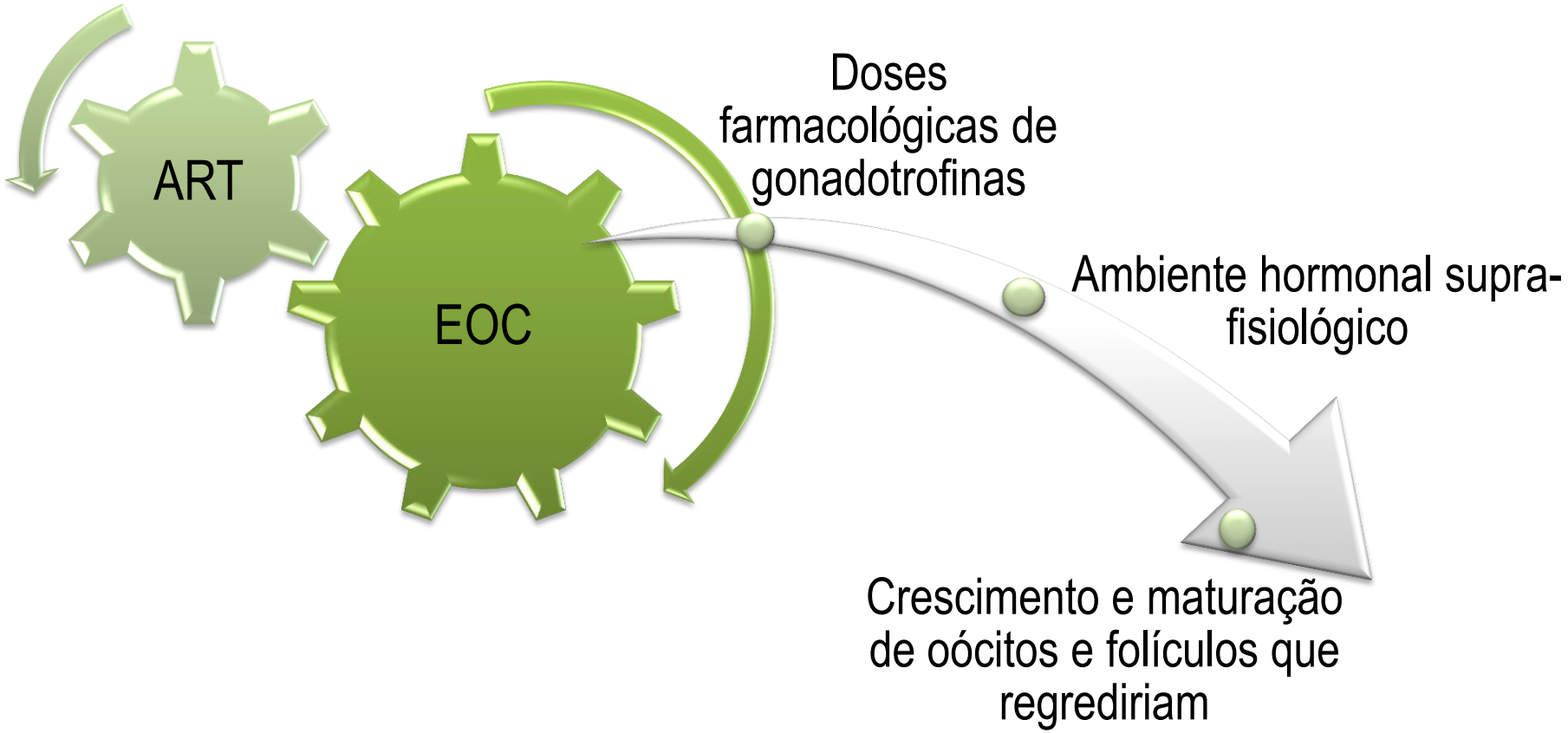
INTRODUÇÃO

✓ Estímulo ovariano controlado



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MATERIAL AND METHODS

- ✓ Statistical analyses

Patient and cycle characteristics

Normality distribution	Shapiro wilk
Group homogeneity	Levenne
Patronization using z score	

Comparisons by ANOVA followed by the Bonferroni post-roc test

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HYPOTHESIS

- The study of signalling molecules, such as miRNAs, in the serum of patients undergoing IVF may be a valuable approach to predict the response to COS and facilitate the development of an individualised gonadotrophin dosing strategy

OBJETIVE

- Identify potential miRNAs biomarkers of ovarian response to COS.

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