Impact of mild ovarian stimulation on implantation

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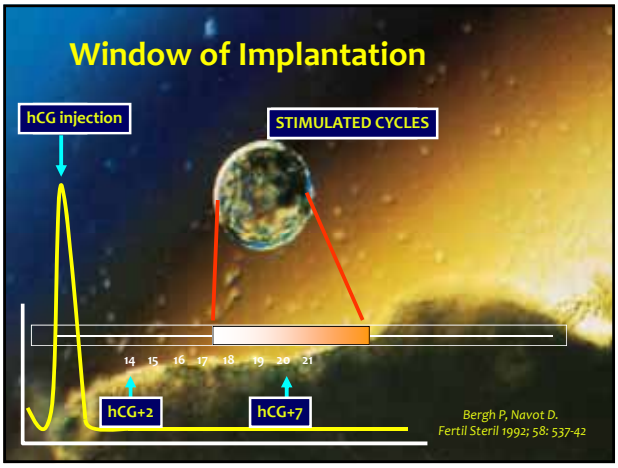
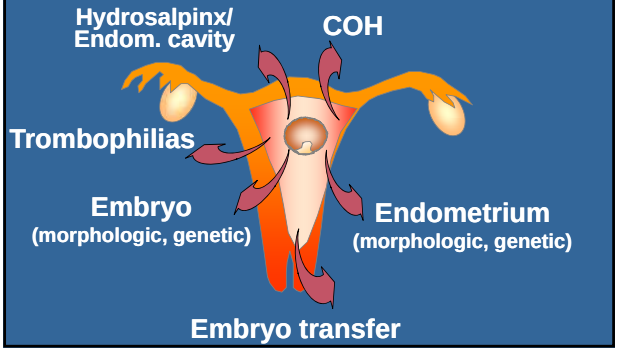
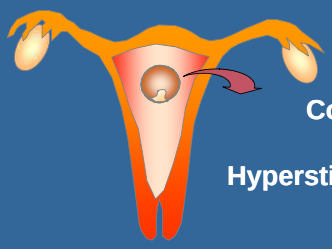
Window of Implantation



Implantation

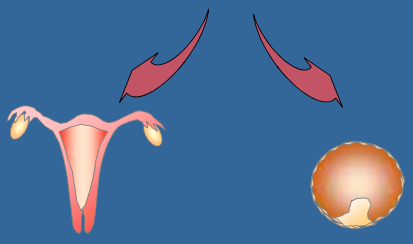


Factors involved



Controlled Ovarian Hyperstimulation

High levels of estradiol

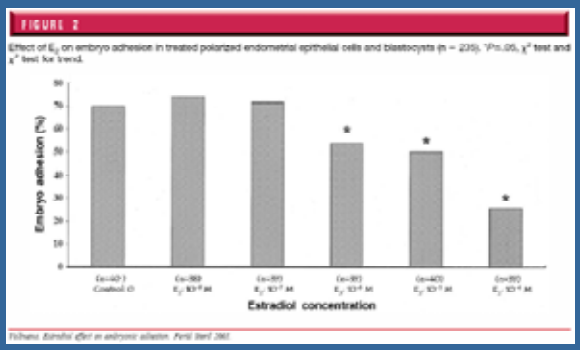


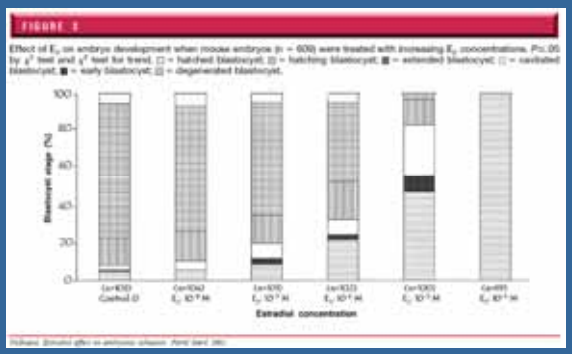
ENDOMETRIUM ?

EMBRYO ?

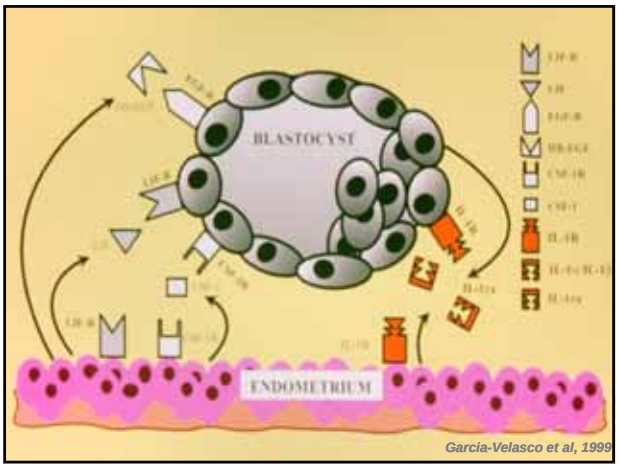
LOW IMPLANTATION RATES IN HIGH RESPONDER PATIENTS

- Clinical studies showing low IR in high responder patients (Pellicer et al. Hum Reprod 1989; Simón C et al. Hum Reprod 1995; Pellicer et al. Fertil Steril 1996; Valbuena et al. Hum Reprod 1999)
- Endometrial receptivity but not embryo quality is affected (Simón C et al. Hum Reprod 1995; Valbuena et al. Hum Reprod 1999)
- Evidence of altered endocrine milieu in the periimplantation period (Pellicer A et al. Fertil Steril 1996)
- Increased IR when E2 levels were lower in subsequent cycles (Simón C et al. Fertil Steril 1998; 70:234-9)
- Extremely high E2 levels are embryotoxic for the embryo (Valbuena et al. Fertil Steril 2001)





- **Receptive endometrium features**
  - » **Morphological markers**
  - » **Biochemical markers**
  - » **Gene expression pattern**



The evolution of technology

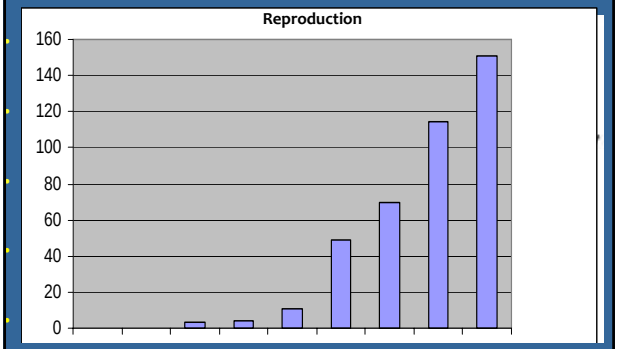
The evolution of technology

1983
3995 US$

2008
300 US$

The evolution of technology

MICROARRAY TECHNOLOGY



1

COH does have an impact on endometrium

EFFECT OF CONTROLLED OVARIAN HYPERSTIMULATION IN IVF ON ENDOMETRIAL GENE EXPRESSION PROFILES

Caucasian
Fertile women with normal cycles
18–35 years
body mass index:19-25 kg/m2

EXPERIMENTAL DESIGN

FIVE WOMEN

Biopsies

1 → LH+2

2 → LH+7

Comparison: NATURAL CYCLE

LH+2 vs LH+7

FIVE WOMEN

Biopsies

1 → LH+7

2 → hCG+7

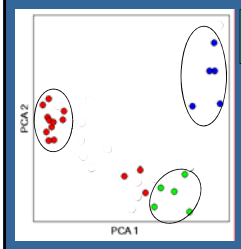
Comparison: COH CYCLE

LH+7 vs hCG+7

Horcajadas et al. Mol Hum Reprod 2005

EFFECT OF CONTROLLED OVARIAN HYPERSTIMULATION IN IVF ON ENDOMETRIAL GENE EXPRESSION PROFILES

PCA ANALYSIS



Comparative results

		LH7 / LH2 DNA fragments FC>2.0	
		Up 894	down 505
hCG+7/LH+7	Up 281	9	115
hCG+7/LH+7	Down 277	227	0

LIST OF THE TEN MOST UP- AND DOWN-REGULATED GENES AT hCG+7 versus LH+7

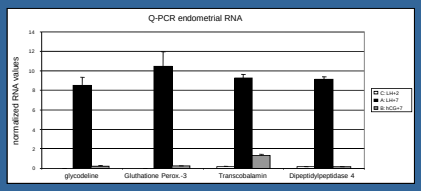
UP

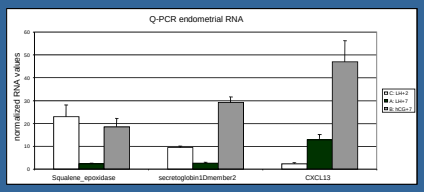
Name	Fold Change	Functional Category
hypothetical protein FLJ22390	38,87	Unknown
Troponin C, show	30,89	Structural protein
matrix metalloproteinase 26	16,96	Enzyme
major histocompatibility complex, class II, DO beta	12,23	Immune response
differentially expressed in hematopoietic lineages	11,89	Inhibitor
Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5	11,88	Inhibitor
Calpain 6	11,80	Glycoprotein
Galanin	11,79	Neuropeptide
Sorbitol dehydrogenase	11,55	Enzyme
Branched chain keto acid dehydrogenase E1, beta polypeptide (maple syrup urine disease)	10,32	Enzyme

DOWN

Name	Fold Change	Funcional Category
Cartilage oligomeric matrix protein (pseudoachondroplasia, epiphyseal dysplasia 1, multiple)	-58,55	Structural protein
dipeptidylpeptidase 4 (CD26, adenosine deaminase complexing protein 2)	-31,25	Immune response
leukemia inhibitory factor (cholinergic differentiation factor)	-23,02	Cytokine
Mucin 16	-13,61	Membrana protein
insulin-like growth factor binding protein 1	-11,99	Regulatory protein
glutathione peroxidase 3 (plasma)	-11,81	Enzyme
solute carrier family 15 (oligopeptide transporter), member 1	-10,62	Transporter
progestagen-associated endometrial protein (placental protein 14, pregnancy-associated endometrial alpha-2-globulin, alpha uterine protein)	-9,83	Secreted protein
ATP-binding cassette, sub-family C (CFTR/MRP), member 3	-9,58	Transporter
Calponin 1, basic, smooth muscle	-9,26	Muscle protein

VALIDATION STUDIES BY QUANTITATIVE-PCR





2

Not all protocols are created equal

Study of the endometrial development in oocyte donors treated with either high- or standard-dose GnRH antagonist compared to treatment with a GnRH agonist or in natural cycles

EXPERIMENTAL DESIGN

Caucasian
Fertile women with normal cycles
18–35 years
body mass index:19-25 kg/m2

Antagonist
Agonist

Biopsies

12	⇨	standard-dose ganirelix	hCG+2 hCG+7
9	⇨	high-dose ganirelix	hCG+2 hCG+7
10	⇨	Buserelin	hCG+2 hCG+7
12	⇨	Natural cycle	LH+2 LH+7

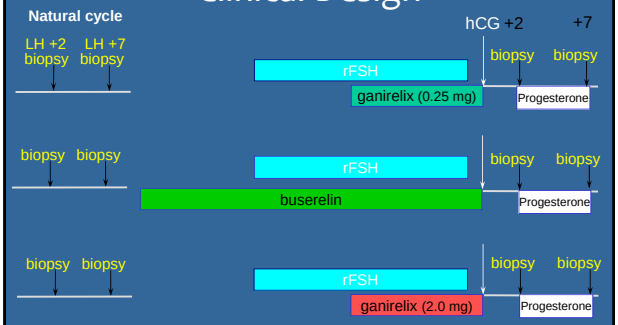
Simón et al. Hum Reprod 2005

Aims of the study

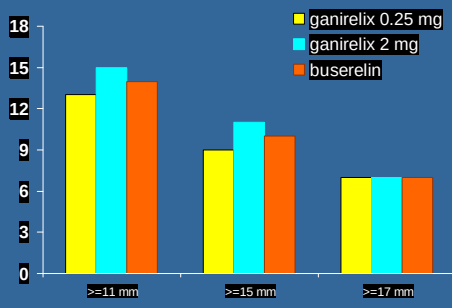
- Number of follicles on the day of hCG
- Serum hormone values at different times of the stimulated cycle
- **Endometrial dating of biopsies by Noyes method**
- Expression of Estradiol and Progesterone receptors (immunohst)
- Scanning electron microscopy
- **Genomic studies by microarray**
- Validation of genomic assays by Q-PCR

Simón et al. (2005) Hum Reprod 12:3318-27

Clinical Design



Median # of follicles on the day of hCG
All Subjects



LH and P rises

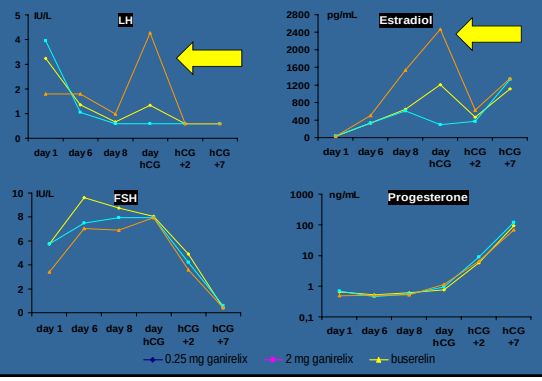
LH ≥ 10 IU/L ; P ≥ 1 ng/mL

	prior		during	
	LH rise	LH+P rise	LH rise	LH+P rise
ganirelix 0.25 mg	0	0	0	0
ganirelix 2 mg	1	1	0	0
buserelin	NA	NA	4	4

	Subject	Day	LH value	P value
ganirelix 2 mg	0027	6	31.0	1.2
buserelin	0008	hCG	10.0	1.14
	0014	hCG	10.8	1.25
	0030	hCG	11.0	2.47
	0035	9	13.8	1.01

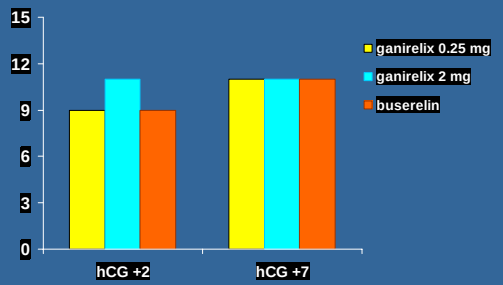
Median serum hormones

Note: days 1-6-8: subjects with 8 days of recFSH



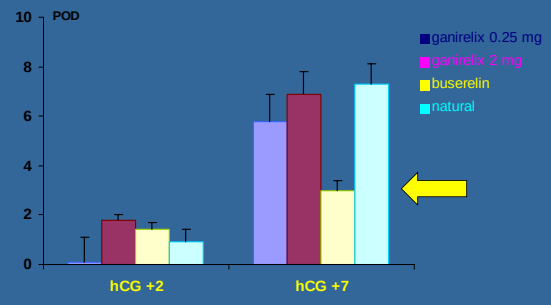
Median endometrial thickness

All Subjects



Endometrial dating

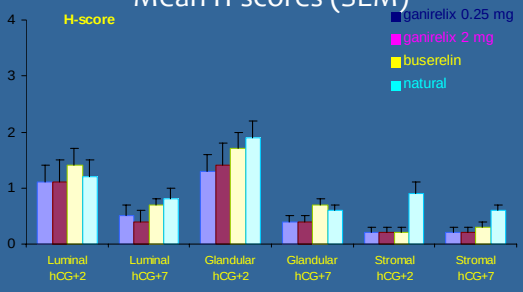
Mean (SEM) - according to Noyes criteria



POD = post ovulatory day

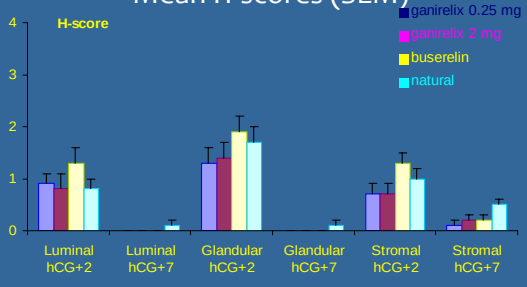
E-receptor (α)

Mean H-scores (SEM)

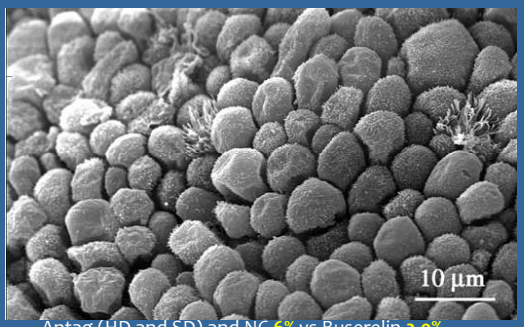


P-receptor (A and B)

Mean H-scores (SEM)



Scanning electron microscopy: sample hCG+7ganirelix (2 mg group)

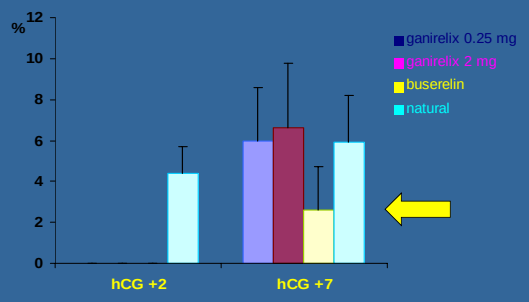


Antag (HD and SD) and NC **6%** vs Buserelin **2.9%**

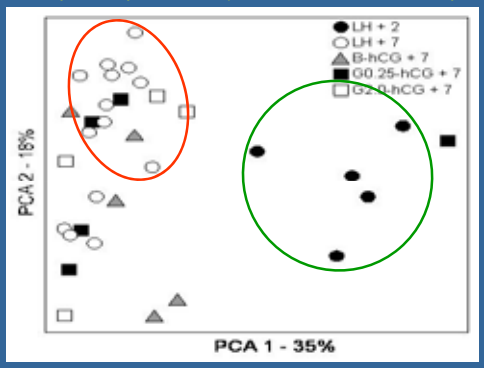
High-dose ganirelix treatment can produce the required epithelial response.

SEM evaluation pinopods

Percentage of cells exhibiting any type of pinopods



Principal Component Analyses of 33 endometrial samples



Using 500 randomly selected genes.

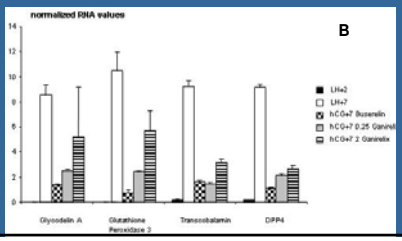
Comparison of the microarray (Panel A) and the Q-PCR (Panel B) data for four selected genes

A

	Average Expression Values				
	Natural cycle		Buserelin	Ganirelix 0.25 mg/day	Ganirelix 2.0 mg/day
Day	LH+2	LH+7	hCG+7	hCG+7	hCG+7
Glycodelin A	64	5002	2733	3199	4694
Glutathione peroxidase 3	134	3943	1959	2387	3574
Transcobalamin	41	1085	148	158	297
DPP4	-34*	408	5	-9*	41

* Negative values indicate absence of expression of the gene as determined by microarray

Negative values indicate absence of expression of this gene as determined by microarray



Comparison of the microarray studies on the gene expression pattern of the stimulated cycles compared to the natural cycles

			WINDOW OF IMPLANATION GENES		
	Treatment and regulation sense	Number of genes	Normally up-regulated (n = 894)	Normally down-regulated (n = 504)	
First STUDY	Leuprolide (agonist)				
	Up	281	9	115	
	Down	277	227	0	
Second STUDY	Ganirelix 0.25 mg/day (antagonist)				
	Up	22	0	4	55%
	Down	69	46	0	
	Ganirelix 2 mg/day (antagonist)				
	Up	88	0	7	21%
	Down	24	15	1	
	Buserelin long protocol (agonist)				
	Up	22	3	4	70%
	Down	100	76	2	

Simón et al. Hum Reprod 2005

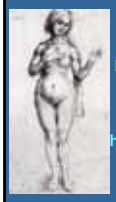
3

Endometrial changes through WOI

GENE EXPRESSION PROFILING DURING THE WINDOW OF IMPLANTATION IN NATURAL versus STIMULATED CYCLES

EXPERIMENTAL DESIGN

50 WOMEN



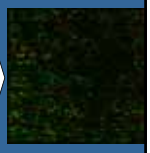
FIVE ENDOMETRIAL BYOPSIES AT EACH TIME POINT

LH Day 1 2 3 4 5 6 7 8 9

hCG Day 1 2 3 4 5 6 7 8 9

PRE-RECEPTIVE RECEPTIVE

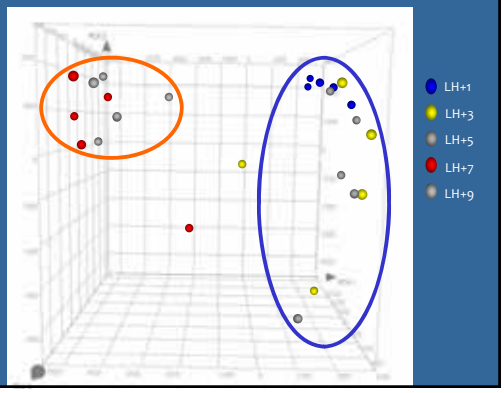
AFFIMETRIX HG-133A >22,000 genes



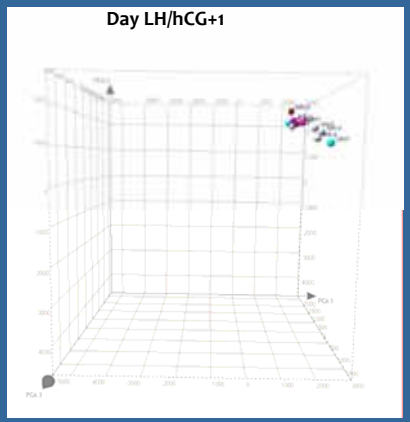
Caucasian
Fertile women with normal cycles
23–39 years
body mass index:19-25 kg/m2

HISTOLOGICAL CONTROL: BLIND DATING BY NOYES CRITERIA

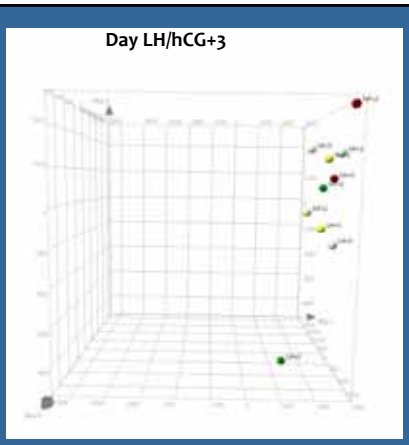
PCA OF THE ENDOMETRIAL SAMPLES FROM LH+1 TO LH+9



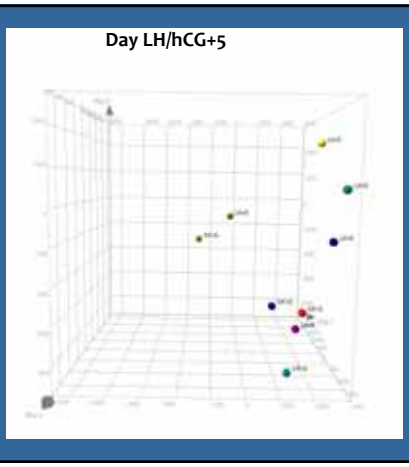
Day LH/hCG+1



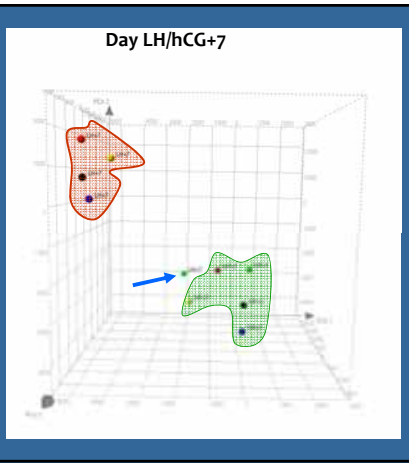
Day LH/hCG+3



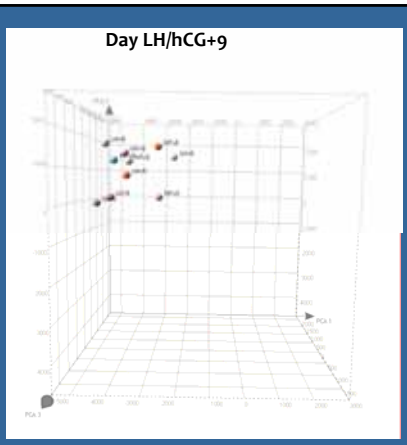
Day LH/hCG+5



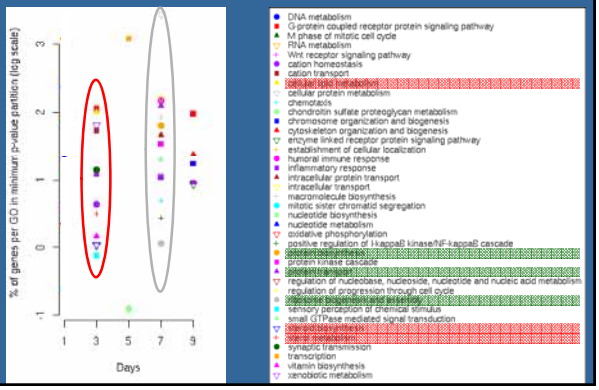
Day LH/hCG+7



Day LH/hCG+9



BIOLOGICAL PROCESSES OVER-EXPRESSED DAILY in NATURAL CYCLES



MOST DIFFERENTIATED FUNCTIONALITIES IN RECEPTIVE ENDOMETRIUM IN NATURAL versus STIMULATED CYCLES

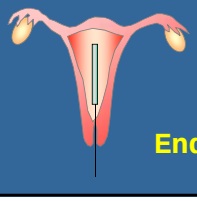
NATURAL CYCLE	STIMULATED CYCLE
GO biological process	**GO biological process**
GO terms over-expressed:	GO terms over-expressed:
1. antigen processing, endogenous antigen via MHC class I 2. antigen presentation, endogenous antigen 3. complement activation, classical pathway 4. response to drug 5. regulation of DNA metabolism 6. mitosis 7. DNA replication 8. small GTPase mediated signal transduction 9. cell division 10. negative regulation of progression through cell cycle 11. skeletal development 12. DNA repair 13. amino acid metabolism 14. cytoskeleton	1. **mitotic checkpoint** 2. antigen processing, endogenous antigen via MHC class I 3. **spindle organization and biogenesis** 4. antigen presentation, endogenous antigen 5. **mitotic sister chromatid segregation** 6. regulation of DNA metabolism 7. **microtubule-based movement** 8. cell division 9. **phosphoinositide-mediated signaling** 10. **DNA-dependent DNA replication** 11. **regulation of development** 12. **nucleotide metabolism** 13. DNA repair 14. **cell proliferation** 15. **regulation of signal transduction** 16. **carboxylic acid metabolism** 17. **positive regulation of cellular process** 18. **negative regulation of cellular physiological process**

Clinical applications

Diagnosis

Gene targeting ➡ Protein analysis

Diagnostic method must be non-invasive and at the time of embryo transfer

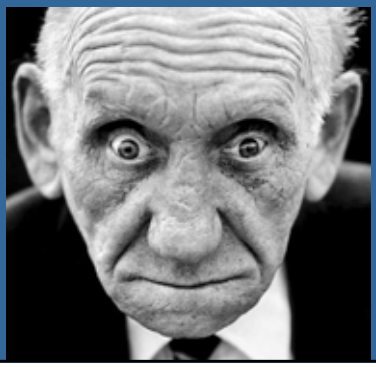


Endometrial fluid

CONCLUSIONS

- Specific gene pattern can be identified daily in the luteal phase by gene expression analysis.
- Biological pathways regulating the shift from pre to receptive endometrium have been identified.
- There are WOI genes, pathways and biological processes disregulated in the receptive endometrium in stimulated versus natural cycles.
- The antagonist regimens resembled more closely natural cycles when compared to agonist regimen

Future strategies



C. Simón
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