# Impacto de la Hiperestimulación Ovárica Controlada sobre el la Expresión Génica del Endometrio durante la Ventana de Implantación

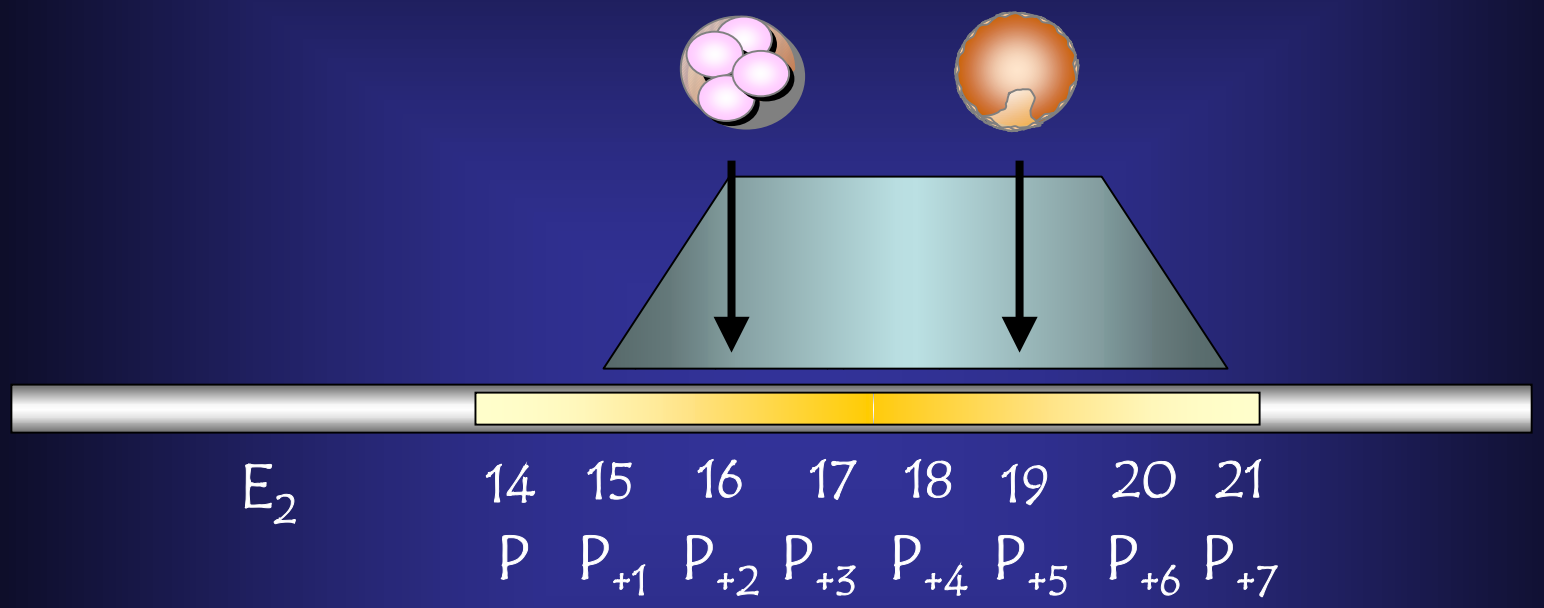
J.A. Horcajadas, A. Riesewijk, A. Cervero, A. Pellicer, S. Mosselman and C. Simón

Instituto Valenciano de Infertilidad (IVI)

Fundación IVI

Universidad de Valencia

# RECEPTIVIDAD ENDOMETRIAL HUMANA

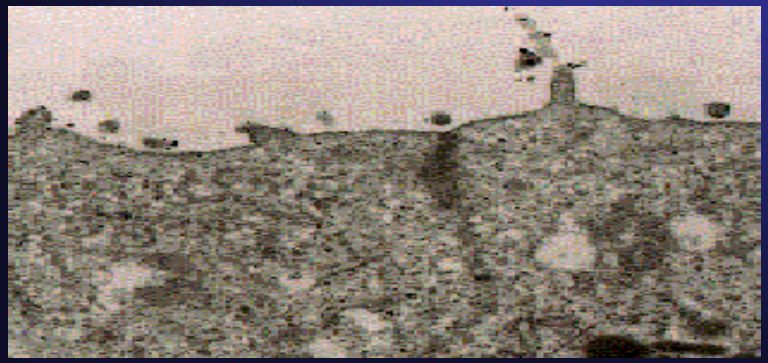


# Características del endometrio receptivo

» Marcadores Morfológicos

» Marcadores Bioquímicos

» **Patrón de Expresión Génica**



# PERFIL DE LA EXPRESIÓN GÉNICA DEL ENDOMETRIO HUMANO RECEPTIVO EN LOS DÍAS LH+2 VS LH+7 MEDIANTE DNA chips

## DISEÑO EXPERIMENTAL

CINCO MUJERES

Caucasiana
Fertiles con ciclos normales
23-39 años
body mass index:19-25 kg/m2

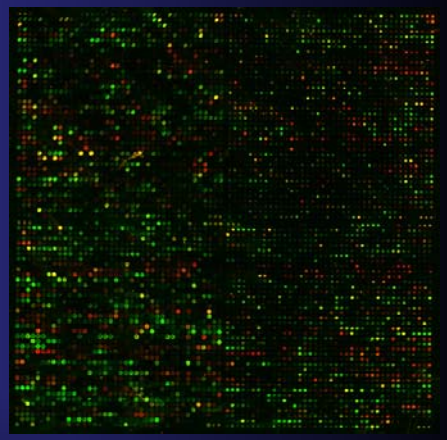
DOS BIOPSIAS ENDOMETRIALES

LH+2

LH+7

RNA AISLAMIENTO

AFFIMETRIX
HG-U95A
12,686 genes



MICROARRAY

Riesewijk et al. Molec Human Reprod 9, 253-64, 2003

# DISEÑOS EXPREIMENTALES PARA ANALIZAR LA RECEPTIVIDAD ENDOMETRIAL HUMANA

**Kao et al.**
11 biopsias
RNA NOT POOLED
Fase Proliferativ VS
Fase Receptiva
AFFIMETRIX HG-U95A
156 Up | Fold >2.0
377 Down
*(Endocrinology)*

**Carson et al.**
6 biopsias
RNA POOLED
LH+(2-4) VS LH+(7-9)
AFFIMETRIX HG-U95A
323 Up | Fold >2.0
370 Down
*(Molec Hum Reprod)*

**Borthwick et al.**
10 biopsias
RNA POOLED
Fase Proliferativa 9-11 VS
LH+(6-8)
AFFIMETRIX HG-U95A-E
90 Up | Fold >2.0
46 Down
*(Molec Hum Reprod)*

**Riesewijk et al.**
10 biopsias
RNA NOT POOLED
LH+2 VS LH+7
AFFIMETRIX HG-U95A
153 Up | Fold >3.0
58 Down
*(Molec Hum Reprod)*

# CONSENSUS GENES: FOLD CHANGE >3

| Accesion number (Function) | Gene name | Riesewijk | Kao | Carson | Borthwick |
|---|---|---|---|---|---|
| **UP-REGULATED GENES PRESENT IN THE FOUR WORKS** | | | | | |
| AF052124 (Structural protein) | Osteopontin | ✓ | ✓ | ✓ | ✓ |
| J02611 (Trasporter) | Apolipoprotein D | ✓ | ✓ | ✓ | ✓ |
| AB020315 (Signalling) | Dickkopf/DKK1 (hdkk-1) | ✓ | ✓ | ✓ | ✓ |
| **UP-REGULATED GENES PRESENT IN THREE OUT OF FOUR WORKS** | | | | | |
| J04129 (Secretory protein) | Placental protein-14/Glycodelin | ✓ | ✓ | | ✓ |
| M31516 (Immnunomodulator) | Decay accelerating factor for complement (CD55, Cromer blood group system) | ✓ | ✓ | | ✓ |
| M84526 (Complement protein) | Adipsin/complement factor D | ✓ | ✓ | | ✓ |
| M55543 (GTP-Binding protein) | Guanylate binding protein 2, interferon-inducible | ✓ | | ✓ | ✓ |
| AB000712 (Receptor) | Claudin 4/CEP-R | ✓ | ✓ | ✓ | |
| AA420624 (Signalling) | Monoamine oxidase A (MAOA) | ✓ | ✓ | | ✓ |
| M60974 (Regulatory protein) | Growth arrest and DNA-damage-inducible protein (gadd45) | ✓ | ✓ | | ✓ |
| AB002365 (Cell death factor) | Nip2 | ✓ | | ✓ | ✓ |
| TOTAL GENES ANALYZED | | 153 | 60 | 120 | 85 |
| **DOWN-REGULATED GENES PRESENT IN THE FOUR WORKS** | | | | | |
| U79299 (Secretory protein) | Olfactomedin-related ER localized protein | ✓ | ✓ | ✓ | ✓ |
| TOTAL GENES ANALYZED | | 58 | 87 | 153 | 40 |

*Horcajadas et al, 2004 J. Reprod. Immunol. 63:41-49*

# ESTRATEGIAS GENÓMICAS ALTERNATIVAS

**- Condiciones No Fertiles: Antiprogestina**

The effect of RU486 on the gene expression profile in an endometrial explant model. Catalano et al. (2003) *Mol. Human Reprod. 9:465-473*

**- Condiciones Subfertiles: Enfermedad**

Expression Profiling of Endometrium from Women with Endometriosis Kao et al. (2003) *Endocrinology 144:2870–2881*

**- Condiciones No fertiles: DIU**

Functional Genomics of Endometrial Receptive using an Interceptive Approach. Horcajadas et al. *En Preparación*

# El impacto de la COH en la receptividad endometrial

- En altas respondedoras a gonadotrofinas, niveles suprafisiológicos de E2 en el día de la administración de hCG es deletéreo para la implantación embrionaria(Simón et al., 1995, 1998, 2003; Pellicer et al., 1996)

- Bajas dosis de E2 mantienen el útero en estado receptivo mientras que altas dosis lo vuelve refractario en ratones (Ma et al., 2003).

- En general la receptividad uterina disminuye durante la COH usada en FIV comparada on los ciclos naturales (Paulson et al., 2000). El útero se encuentra avanzado desde el punto de vista histológico.

# OBJETIVO: ESTUDIAR EL EFECTO DE LA COH EN LOS PERFILES DE EXPRESIÓN GÉNICA ENDOMETRIAL

## DISEÑO EXPERIMENTAL

CaucasianA
Fertiles con ciclos normales
23–39 años
body mass index:19-25 kg/m2

**CINCO MUJERES**

Biopsias

1 → LH+2

2 → LH+7

Comparación: CICLO NATURAL

LH+2 vs LH+7

**CINCO MUJERES**

Biopsias

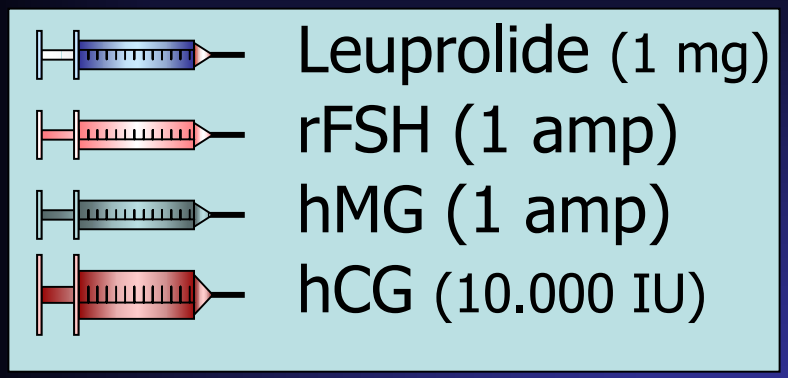
1 → LH+7

2 → hCG+7

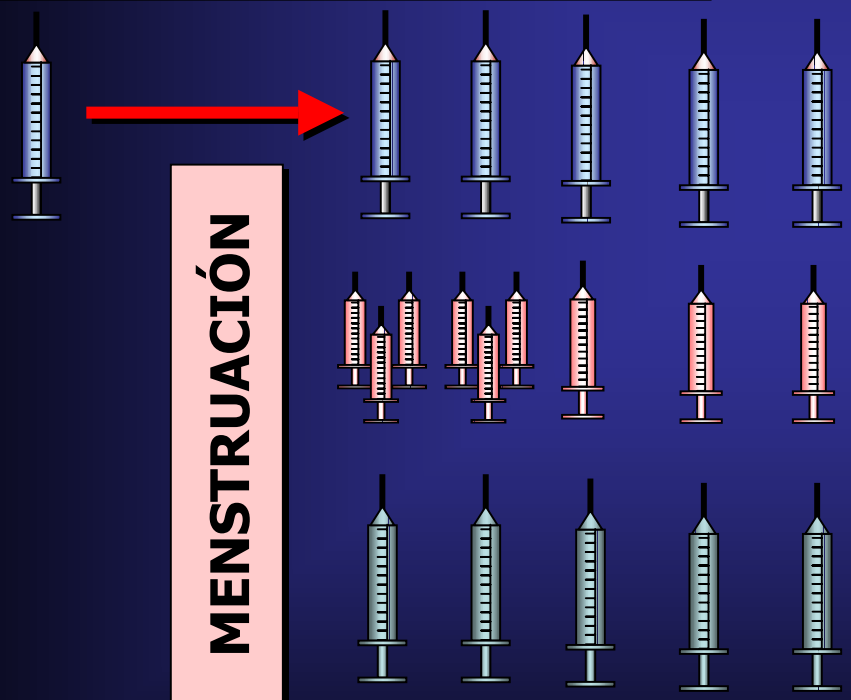
Comparación: CICLO ESTIMULADO

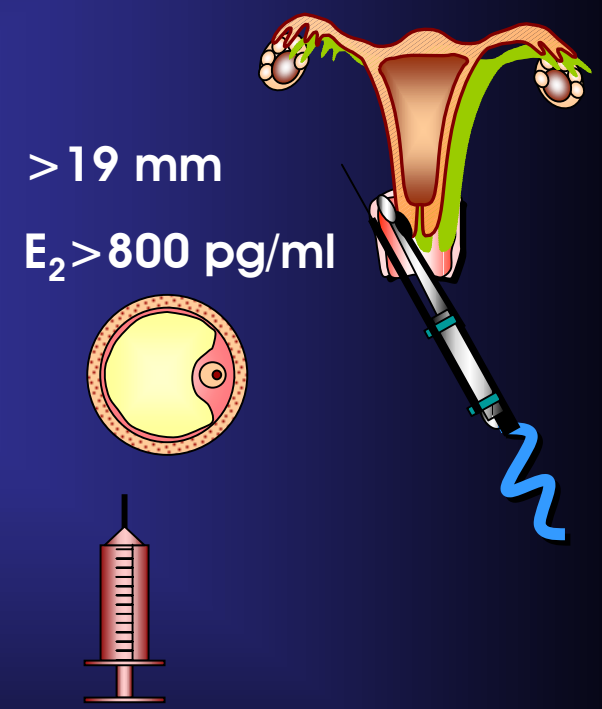
LH+7 vs hCG+7

# PROTOCOLO DE ESTIMULACIÓN




## LONG PROTOCOL




**Día 20..... 1 2 3 4 5 6 7... hCG+7**

# COMPARACIÓN LH+7 vs LH+2 EN EL NUEVO GENECHIP HG_U133A

| Title | Fold Change |
|---|---|
| S100 calcium binding protein P | -100,00 |
| progestagen-associated endometrial protein (placental protein 14, pregnancy-associated endometrial alpha-2-globulin, alpha uterine protein) | -81.61 |
| cartilage oligomeric matrix protein (pseudoachondroplasia, epiphyseal dysplasia 1, multiple) | -77.88 |
| dipeptidylpeptidase 4 (CD26, adenosine deaminase complexing protein 2) | -53.21 |
| ATP-binding cassette, sub-family C (CFTR/MRP), member 3 | -43.36 |
| clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J) | -40.92 |
| nicotinamide N-methyltransferase | -40.8 |
| decay accelerating factor for complement (CD55, Cromer blood group system) | -38.11 |
| **leukemia inhibitory factor (cholinergic differentiation factor)** | **-36.62** |
| defensin, beta 1 | -35.21 |
| unknown | -34.54 |
| ATP-binding cassette, sub-family C (CFTR/MRP), member 3 | -32.17 |
| glutathione peroxidase 3 (plasma) | -30.75 |
| leiomodin 1 (smooth muscle) | -29.73 |
| decay accelerating factor for complement (CD55, Cromer blood group system) | -28.74 |
| transcobalamin I (vitamin B12 binding protein, R binder family) | -27.96 |
| dipeptidylpeptidase 4 (CD26, adenosine deaminase complexing protein 2) | -27.40 |
| unknown | -25.67 |
| growth arrest-specific 1 | -24.79 |
| thrombomodulin | -23.92 |
| solute carrier family 15 (oligopeptide transporter), member 1 | -23.86 |
| transmembrane 4 superfamily member 3 | -23.55 |
| interferon stimulated gene 20kDa | -22.60 |
| monoamine oxidase A | -21.29 |
| glutathione peroxidase 3 (plasma) | -21,00 |
| Unknown | -20.62 |
| complement component 4 binding protein, alpha | -19.50 |

# LISTA DE LOS DIEZ GENES MÁS UP- Y DOWN-REGULATED EN hCG+7 versus LH+7

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 |
| | | |
| **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 |
| | | |
| **ATP-binding cassette, sub-family C (CFTR/MRP), member 3** | -9,58 | Transporter |
| **Calponin 1, basic, smooth muscle** | -9,26 | Muscle protein |

UP

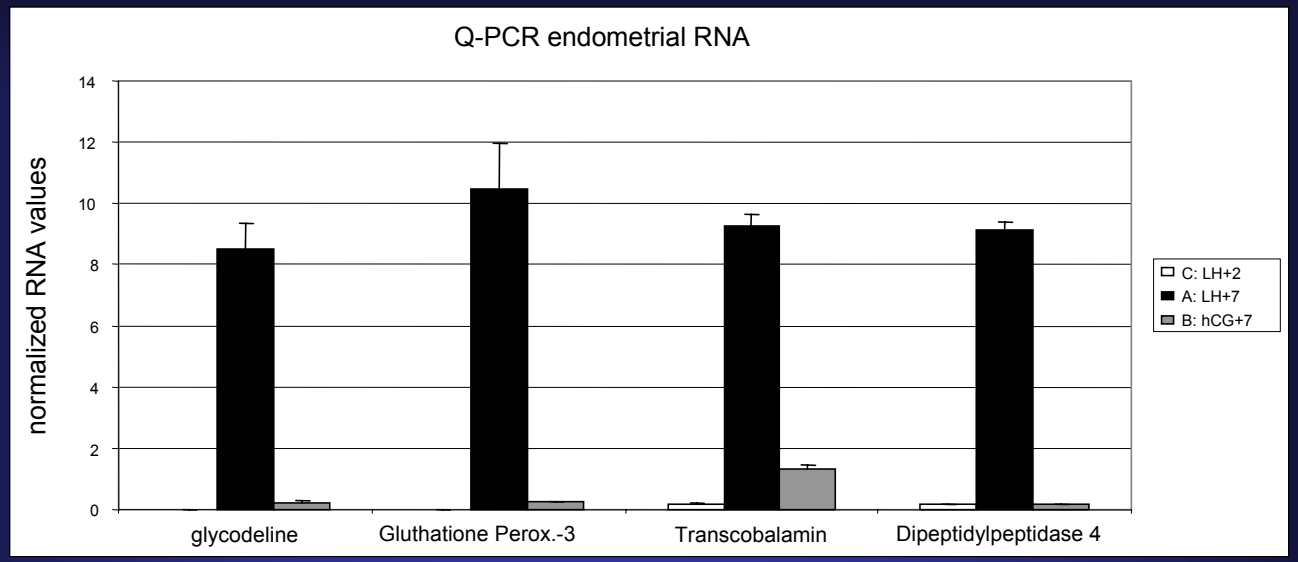
| Name | Fold Change | Funtional Category |
|---|---|---|
| hypothetical protein FLJ22390 | 38,87 | Unknown |
| Troponin C, show | 30,89 | Structural protein |
| matrix metalloproteinase 26 | 16,96 | Enzyme |
| **major histocompatibility complex, class II, DO beta** | 12,23 | Immune response |
| differentially 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 |

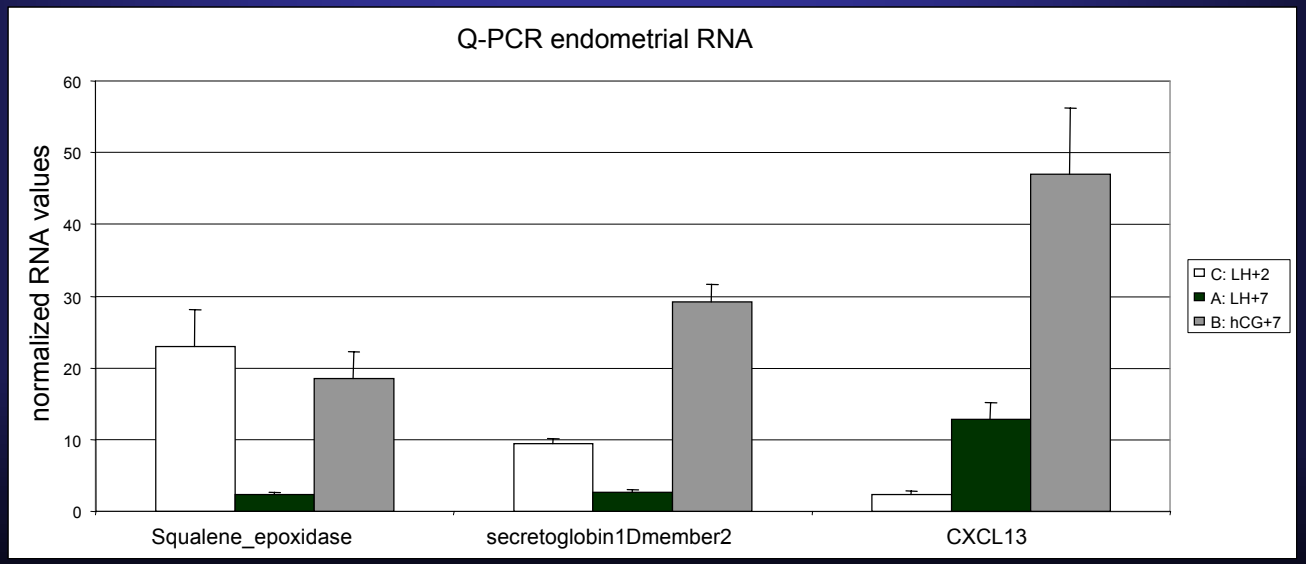
# EFFECTO DE LA COH EN FIV SOBRE LOS PERFILES DE EXPRESIÓN GÉNICA ENDOMETRIAL

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

Horcajadas et al *Mol. Human Reprod.* In press

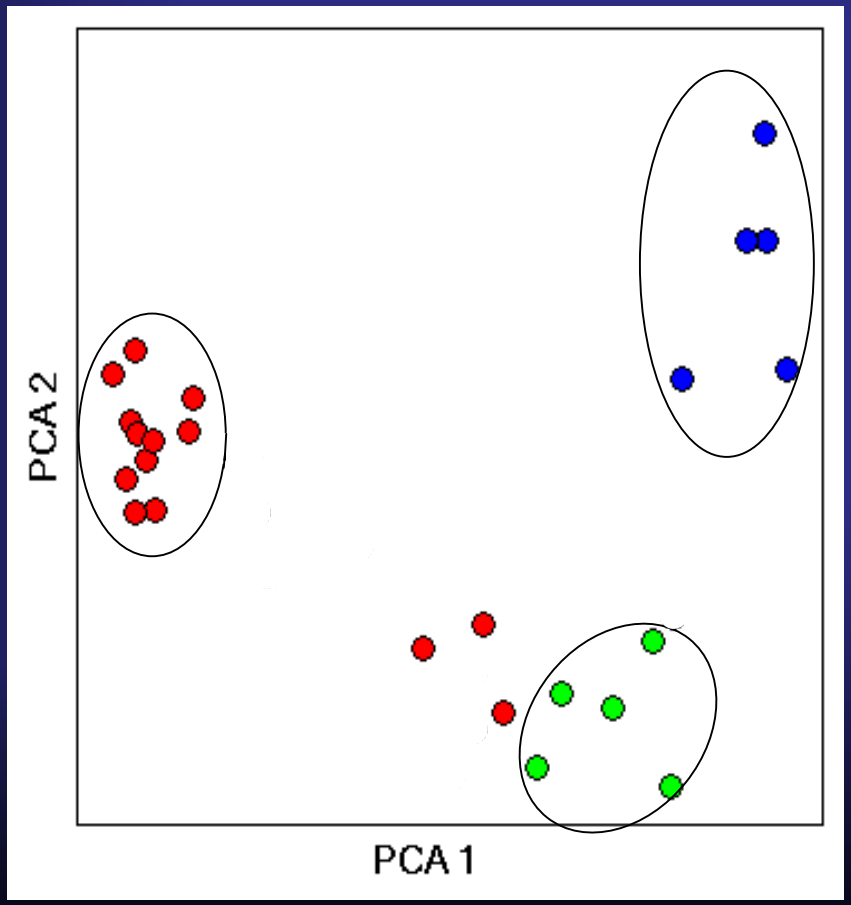
# ESTUDIOS DE VALIDACIÓN POR PCR CUANTITATIVA



# EFFECTO DE LA COH EN FIV SOBRE LOS PERFILES DE EXPRESIÓN GÉNICA ENDOMETRIAL

## ANALISIS POR PCA




LH+2
LH+7
hCG+7

Horcajadas et al
*Mol. Human Reprod.* In press

# CONCLUSIONES

- Hay un alto número de genes relacionados con receptividad endometrial que se encuentran aberrantemente expresados en los ciclos de COH (342 genes), mostrando niveles de expresión más similares a un endometrio no receptivo

- Los protocolos actuales de COH no son suficientemente eficientes para reproducir el status natural de la receptividad endometrial

# FUTURO

- Modificar los tratamientos de COH para obtener protocolos que logren un endometrio que simule, morfológica y funtional-mente el ciclo natural del endometrio con mayor eficiencia y que mejore los niveles de éxito en implantación embrionaria

-Analizar, mediante estudios funcionales, los genes obtenidos en este y otros estudios similares, para determinar su implicación en la receptividad endometrial e implantación

## Administration

Carmen Rodríguez

Jaime Herrero

## Directors

Dr. Carlos Simón

Dr. Antonio Pellicer

fundación IVI

## Embryo Implantation

Dr. José A. Horcajadas

Dr. Francisco Domínguez

Ana Cervero

Isabel Alonso

## PGD

Dr. Julio Martín

Alicia Zurita

## OHSS

Raúl Gómez

## Adult Stem Cell

Irene Carvallo

José A. Martínez

## Embryonic Stem Cells

Dr. M. Angeles Asensi

Dr. Amparo Galán

Dr. Diana Valbuena

Eva Sánchez

M. Carmen Escobedo

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IVI Valencia

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-Anne Riesewijk and Sietse Mosselman,
Organon, Oss (The Netherlands)



Dr. Carlos Simón

csimon@interbook.net

Dr. José A. Horcajadas

jhorcajadas@ivi.es