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ON

OPTIMIZING INFERTILITY TREATMENTS

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The role of the endometrium in implantation in human beings

Prof. Antonio Pellicer

OBJECTIVES

- *To review the molecular basis of implantation*
- *To analyze the clinical relevance of the endometrium in implantation*
- *To discuss the screening methods of endometrial receptivity*
- *To improve IVF outcome through enhanced endometrial receptivity*

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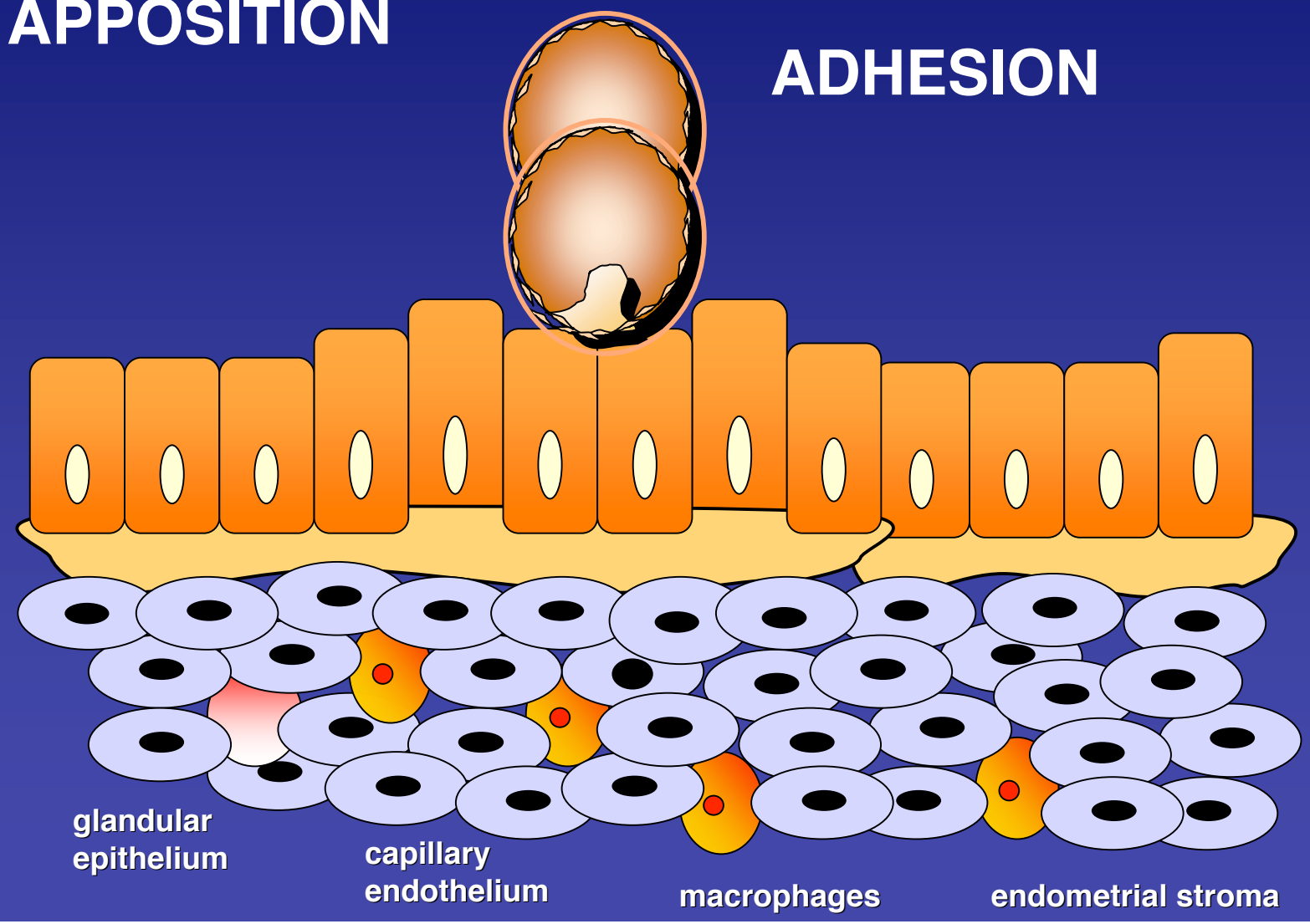
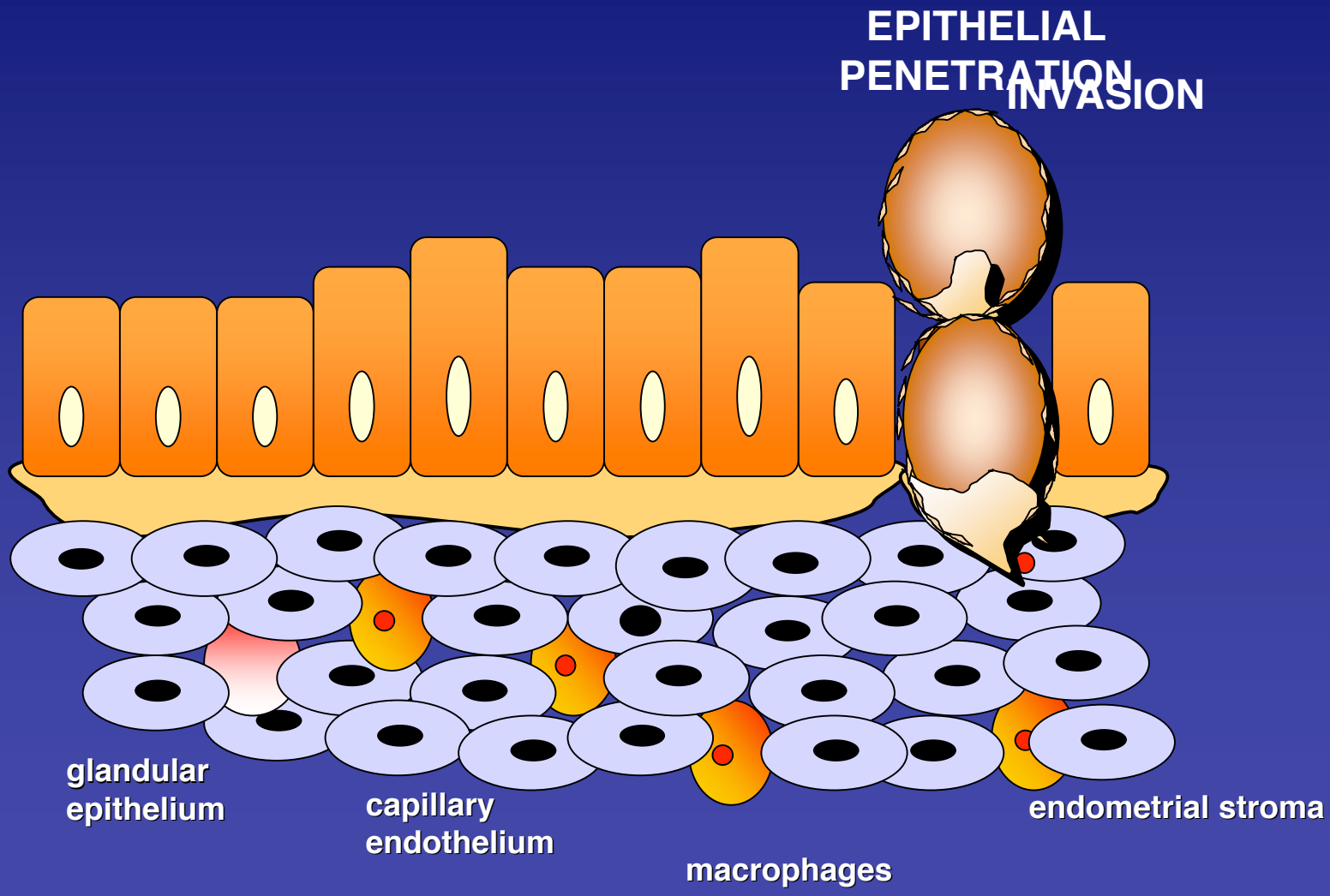
THE IMPLANTATION PROCESS



THE IMPLANTATION PROCESS



MOLECULAR BASIS OF IMPLANTATION

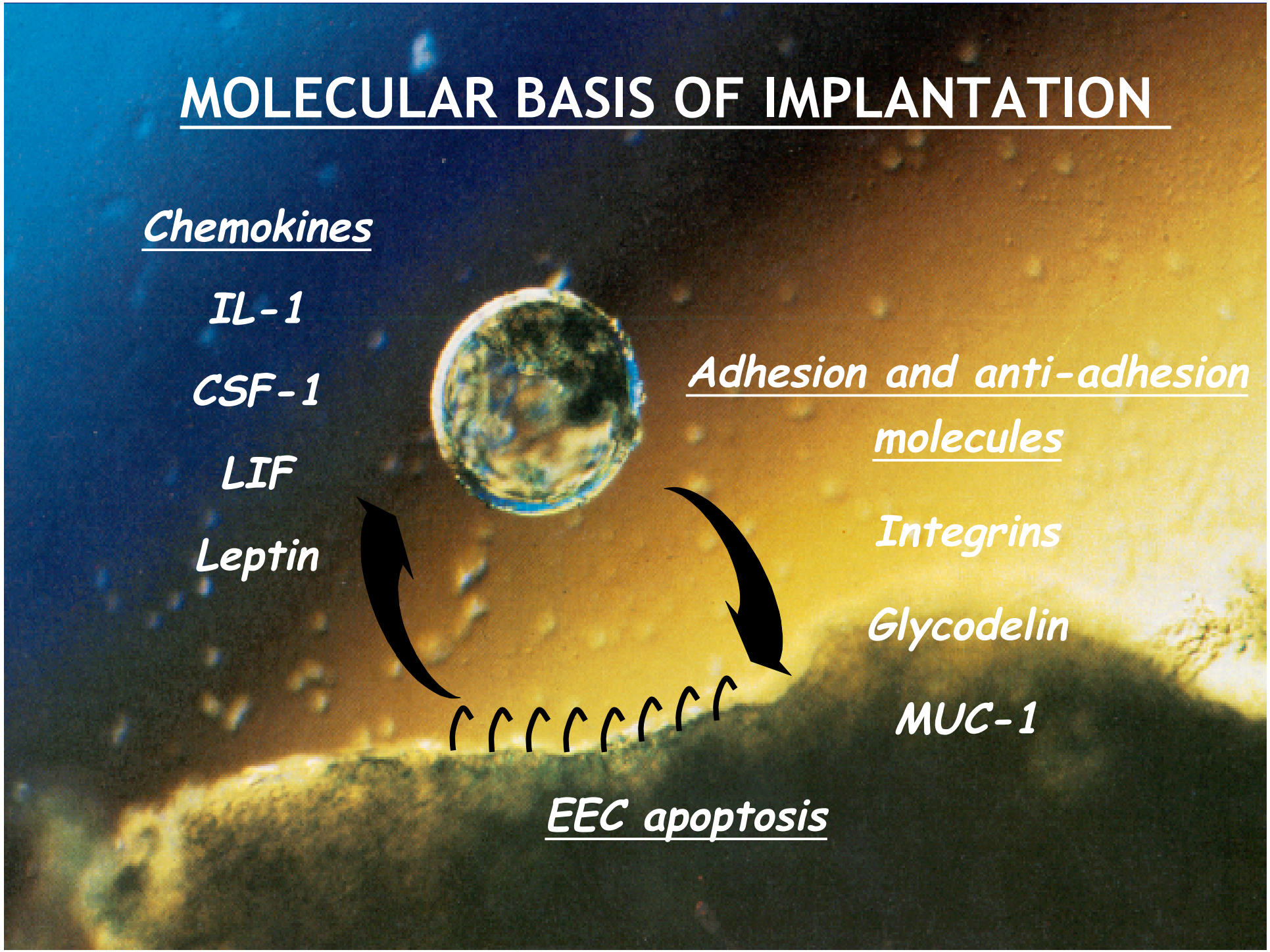
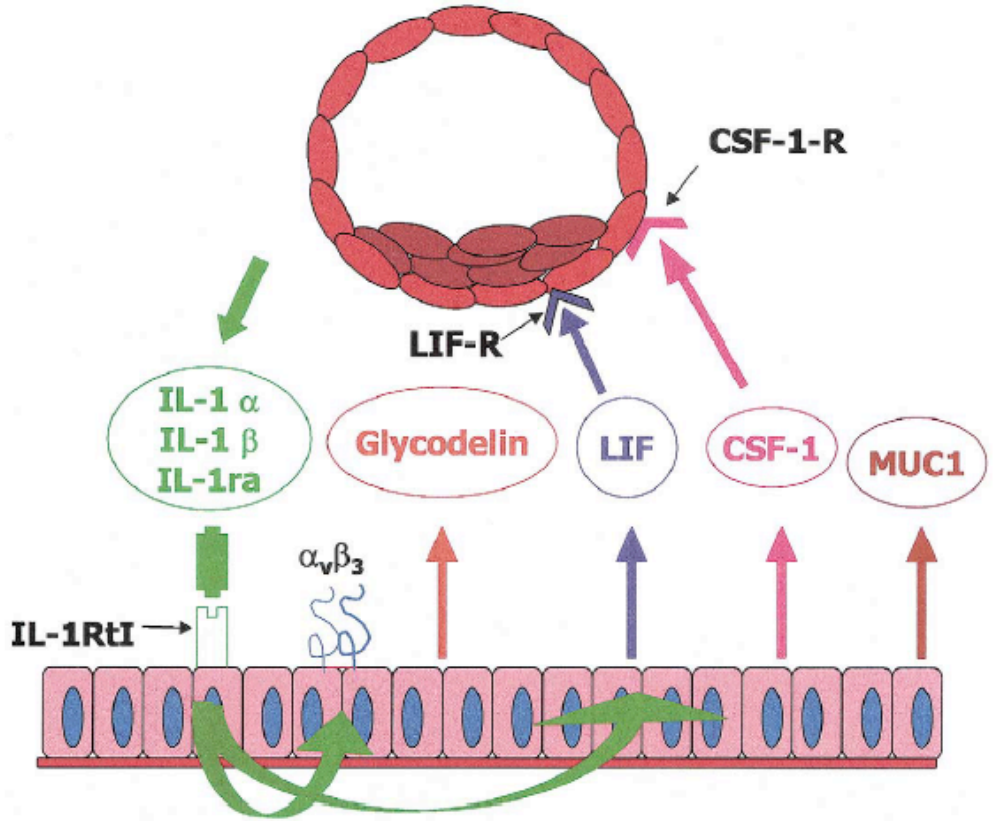


FIGURE 2

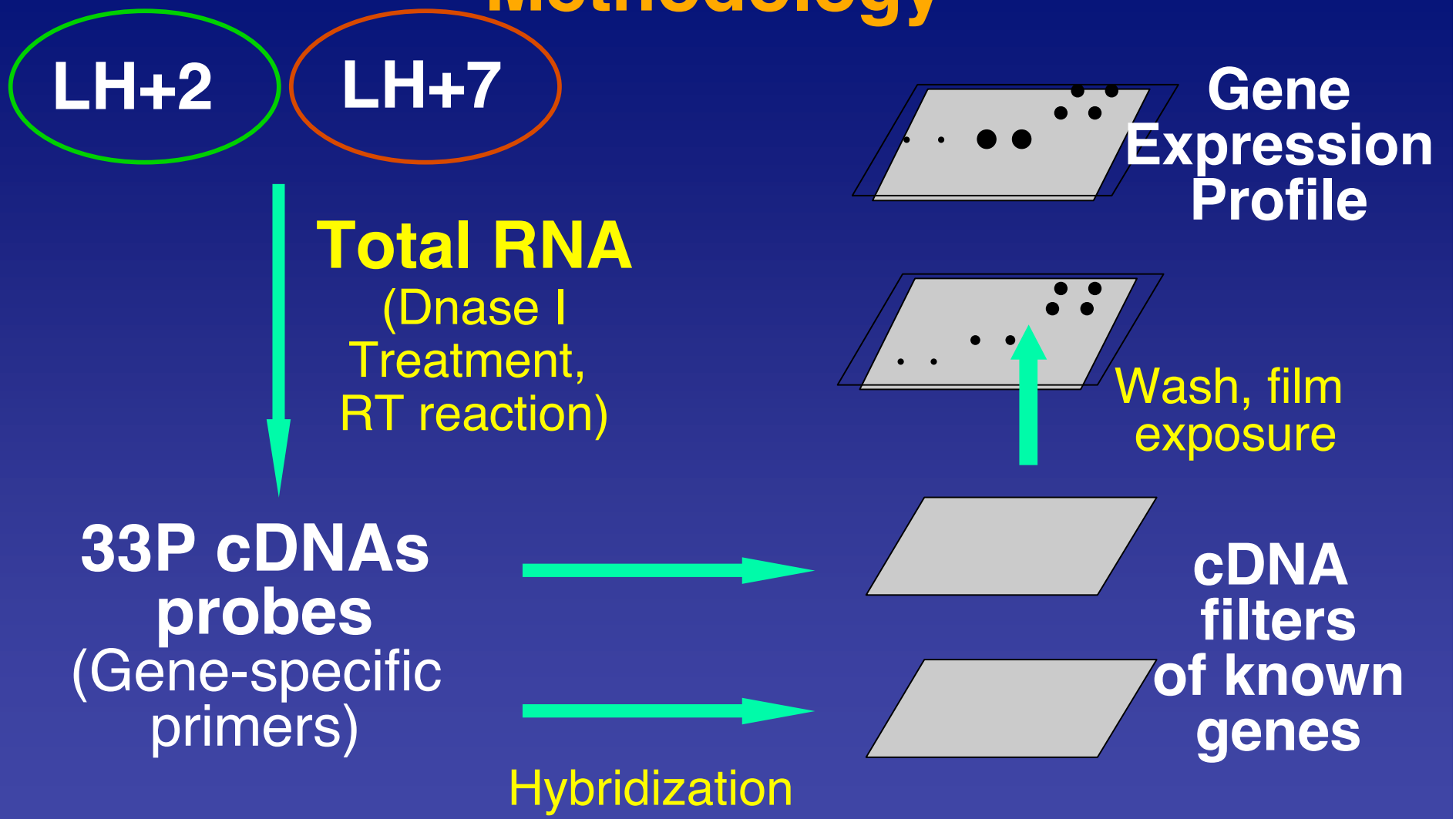
Localization of factors of importance to the interaction between the human blastocyst and endometrium before implantation: interleukin-1α and β (IL-1α, IL-1β), interleukin 1 receptor type 1 (IL-1Rt1) and interleukin 1 receptor antagonist (IL-1ra), leukemia inhibitory factor (LIF) and the receptor (LIF-R), colony-stimulating factor-1 (CSF-1), CSF-1-receptor (CSF-1-R), glycodelin, integrin $\alpha_v\beta_3$, and the polymorphic glycoprotein MUC1. Embryonic secretion of IL-1 induces localized changes in the endometrial luminal epithelium, and the binding of IL-1 to the IL-R on the luminal epithelium may up-regulate the expression of $\alpha_v\beta_3$ and LIF. Adhesion is facilitated by the interaction between glycodelin, LIF, CSF-1, and the corresponding embryonic receptors. The blastocyst may also induce a cleavage of endometrial epithelial MUC1 at the implantation site, thereby making a small zone of the luminal epithelium adhesive, while the remaining parts continue to be nonadhesive.



Lindhard. Endometrial function at implantation. Fertil Steril 2002.

Lindhard et al, Fertil Steril 2002; 78: 221

Methodology



Dominguez et al. J Clin Endocrinol Metab 2003; 88:1849-55: 375 genes
Riesewijk et al. Mol Hum Reprod 2003; 9: 1-13: 14,000 genes

Genes regulated during human endometrial receptivity

	Up at LH+7	Down at LH+7
Strong (>10)	22	5
Medium (5-10)	47	12
Weak (3-5)	84	41
	153	58

EXPERIMENTAL APPROACHES TO ANALYZE HUMAN ENDOMETRIAL RECEPTIVITY

Kao et al.

11 SAMPLES

RNA NOT POOLED

Proliferative phase VS

Receptive phase

AFFIMETRIX HG-U95A

156 Up

377 Down

Fold >2.0

(Endocrinology)

Carson et al.

6 SAMPLES

RNA POOLED

LH+(2-4) VS LH+(7-9)

AFFIMETRIX HG-U95A

323 Up

370 Down

Fold >2.0

(Molec Hum Reprod)

Borthwick et al.

10 SAMPLES

RNA POOLED

Proliferative 9-11 VS LH+(6-8)

AFFIMETRIX HG-U95A-E

90 Up

46 Down

Fold >2.0

(Molec Hum Reprod)

Riesewijk et al.

10 SAMPLES

RNA NOT POOLED

LH+2 VS LH+7

AFFIMETRIX HG-U95A

153 Up

58 Down

Fold >3.0

(Molec Hum Reprod)

CONSENSUS GENES: FOLD CHANGE >3

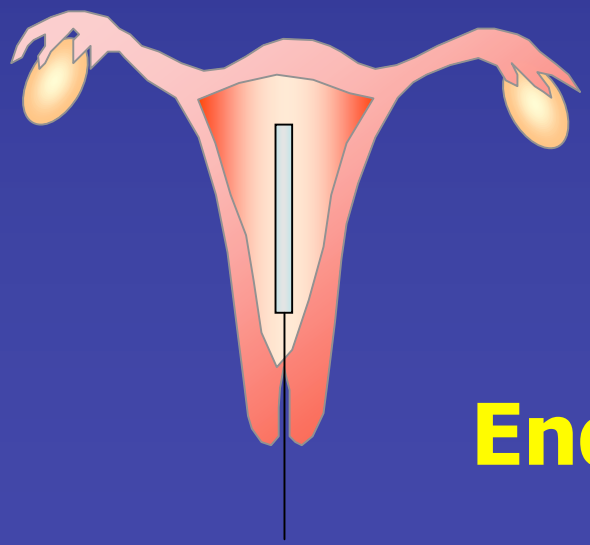
Accesion number (Function)	Gene name	Riesewijk	Kao	Carson	Borthwick
UP-REGULATED GENES PRESENT IN THE FOUR WORKS					
AF052124 (Structural protein)	Osteopontin	✓	✓	✓	✓
J02611 (Trasporter)	Apolipoprotein D	✓	✓	✓	✓
AB020315 (Signalling)	Dickkopf/DKK1 (hdkk-1)	✓	✓	✓	✓
UP-REGULATED GENES PRESENT IN THREE 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

Clinical applications

Diagnosis

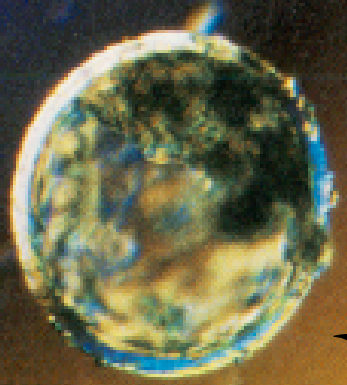
Gene targeting ➡ Protein analysis

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



Endometrial fluid

EMBRYONIC REGULATION OF ENDOMETRIAL MOLECULES



Chemokines

De los Santos et al, BOR 1996

Simón et al, JCEM 1997, 1999

Adhesion and anti-adhesion molecules

Martin et al, BOR 2001

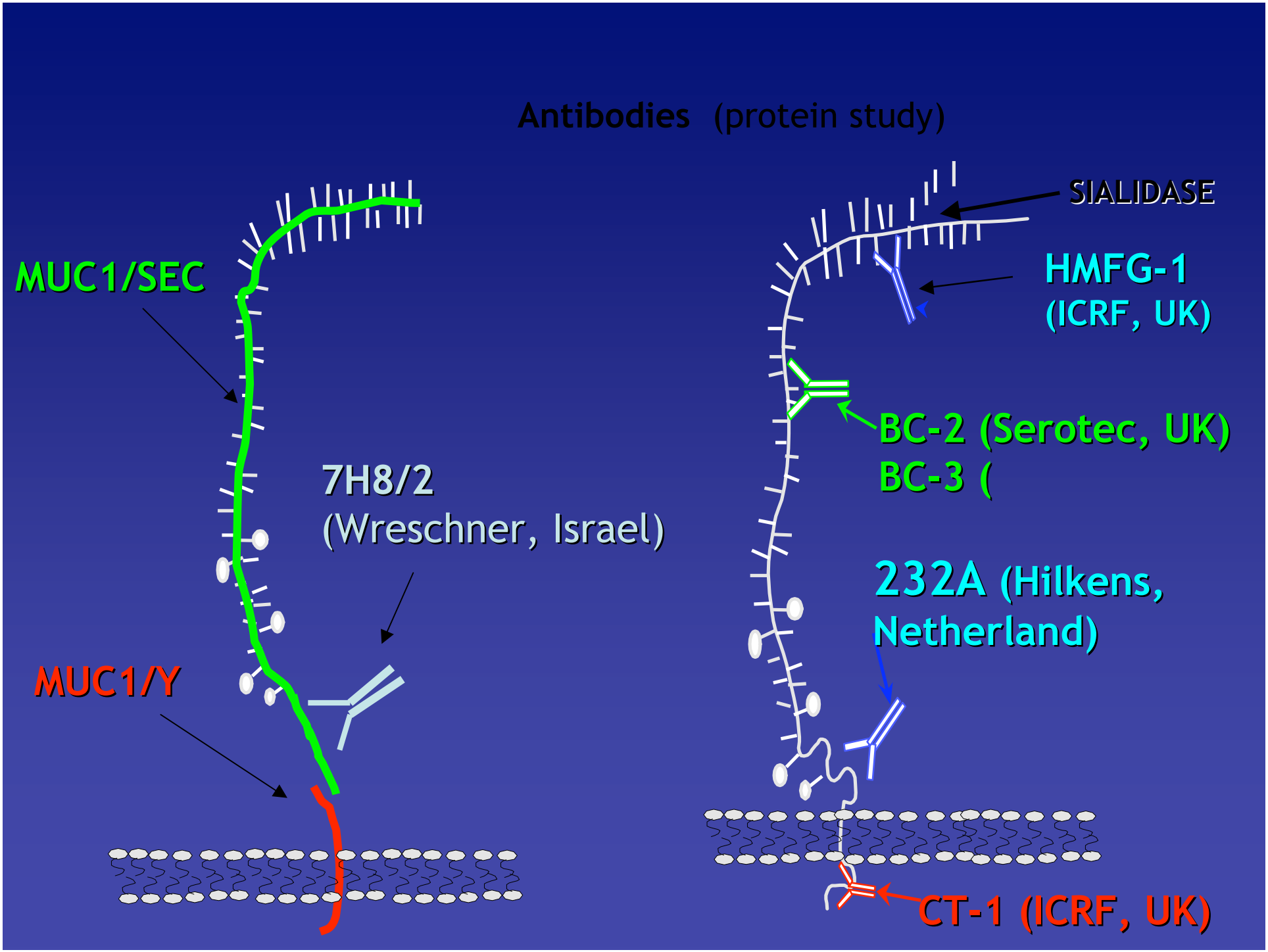
Meseguer et al, BOR 2001

Leptin

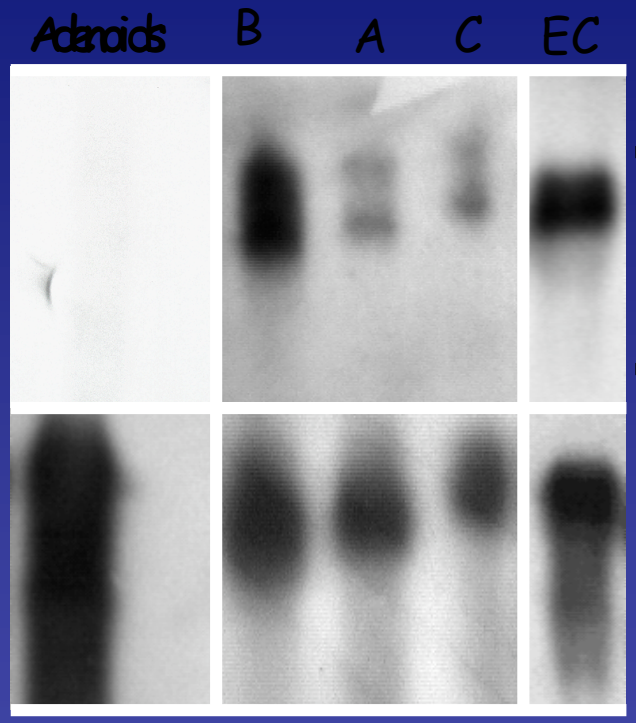
González et al, JCEM 2000

EEC apoptosis

Galán et al, BOR 2001

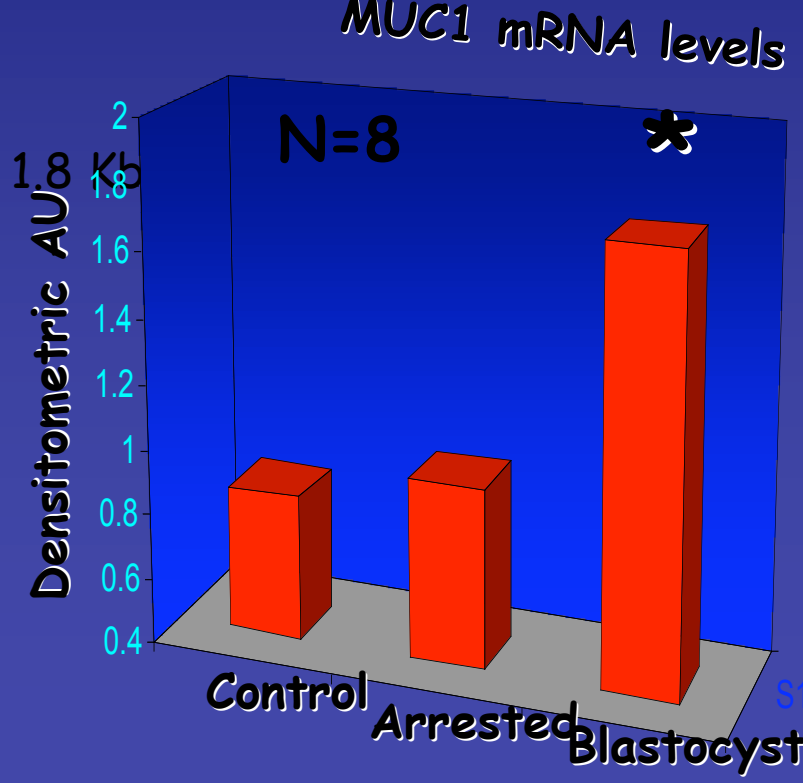

Antibodies (protein study)
MUC1/SEC
7H8/2
(Wreschner, Israel)
MUC1/Y
SIALIDASE
HMFG-1
(ICRF, UK)
BC-2 (Serotec, UK)
BC-3 (
232A (Hilkens,
Netherland)
CT-1 (ICRF, UK)

EMBRYONIC REGULATION OF MUC1 mRNA



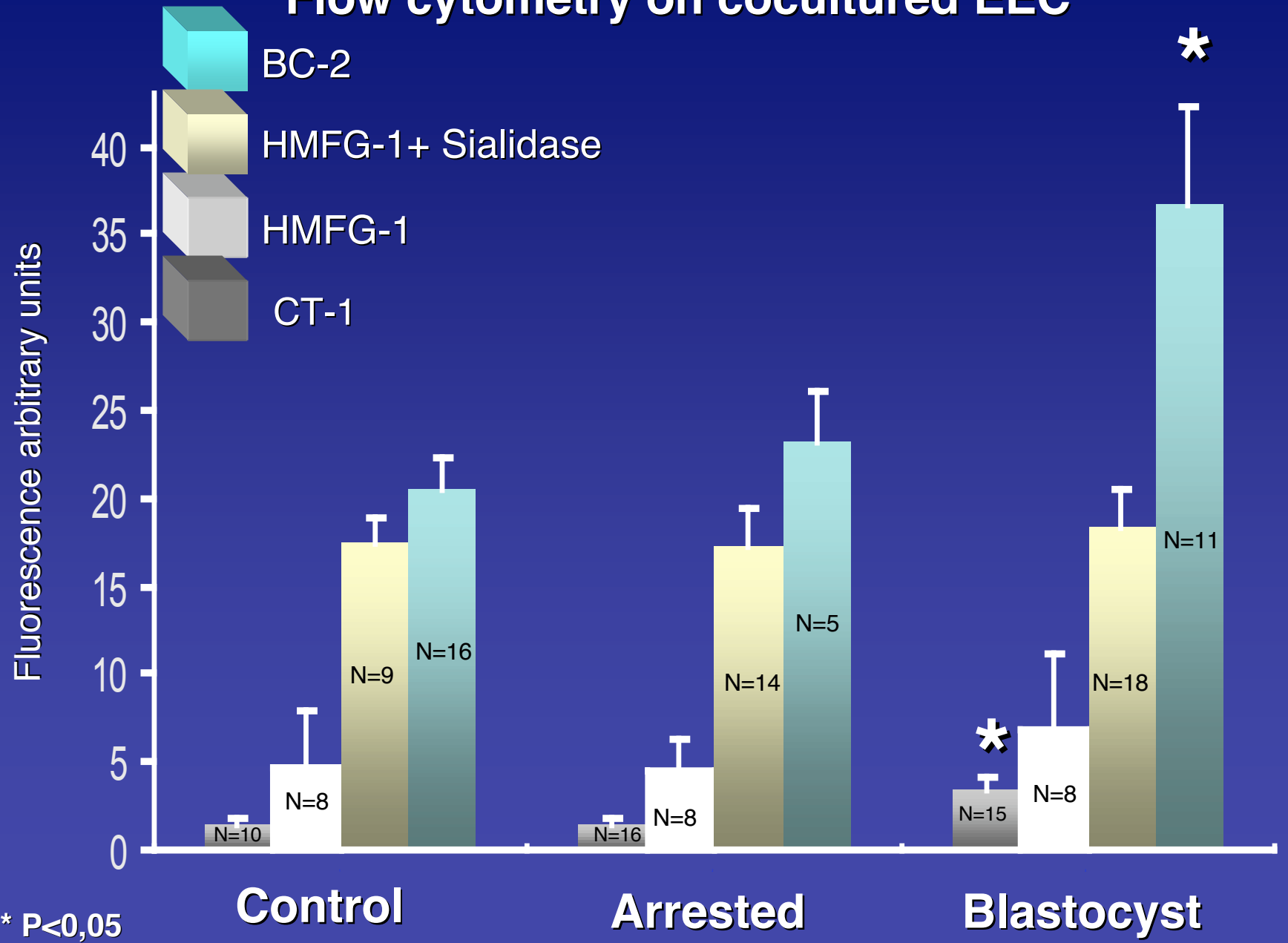
MUC1 mRNA Hybridization signal

28s rRNA Hybridization signal



Results

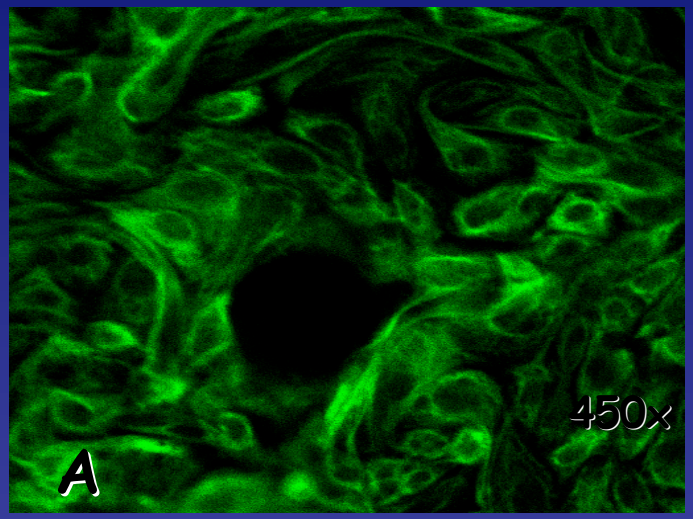
Flow cytometry on cocultured EEC

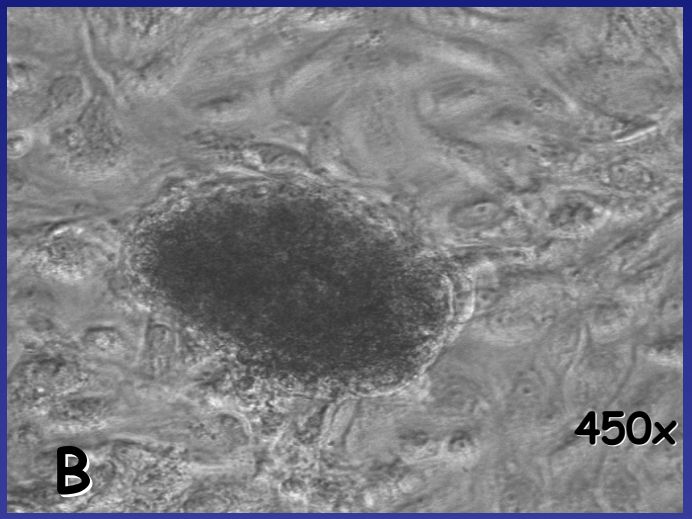


* P<0,05

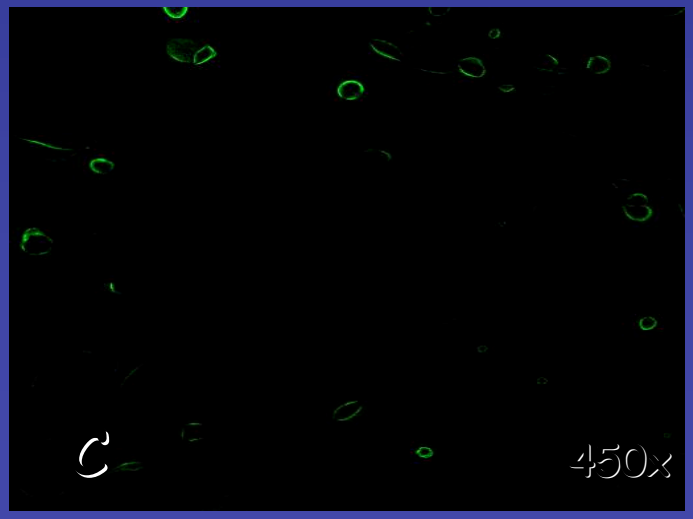
CONFOCAL MICROSCOPY OF HUMAN BLASTOCYST ATTACHMENT

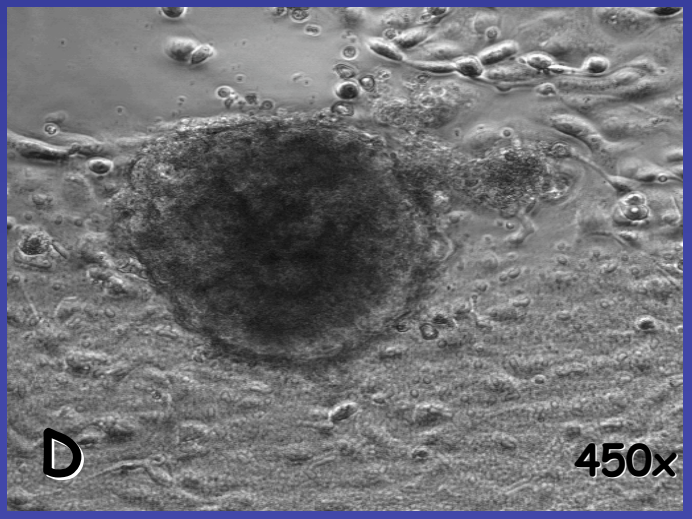
Cytokeratin FITC





MUC1 FITC

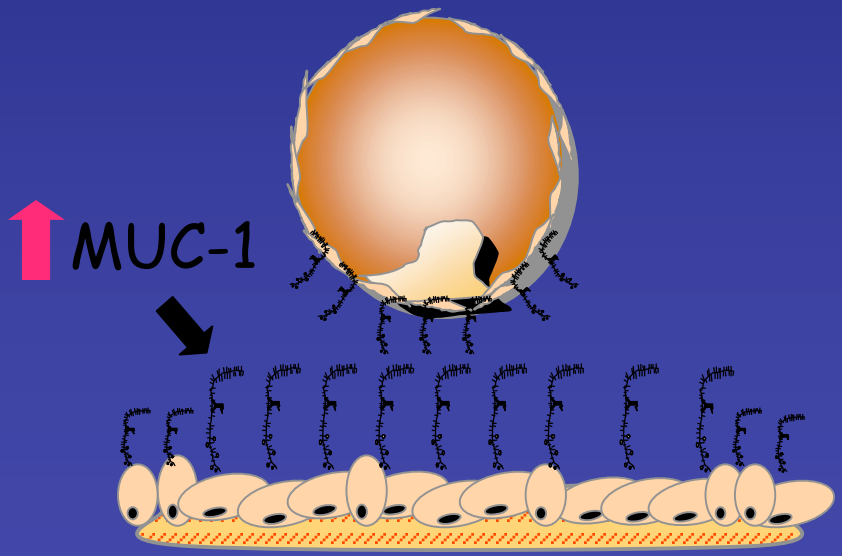




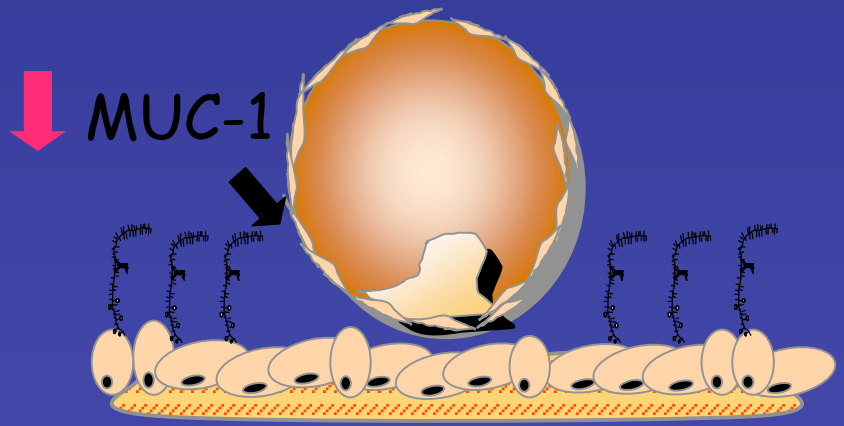
CONCLUSIONS

The human blastocyst induces a coordinated regulation of EEC MUC-1 during aposition and adhesion

Aposition



Adhesion



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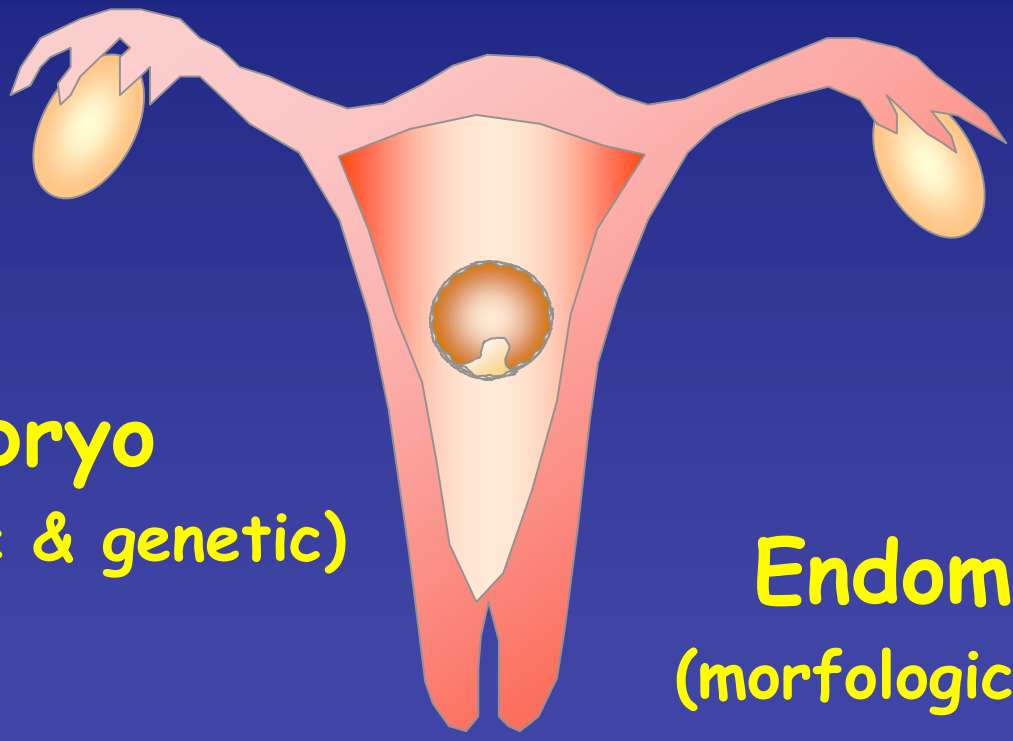
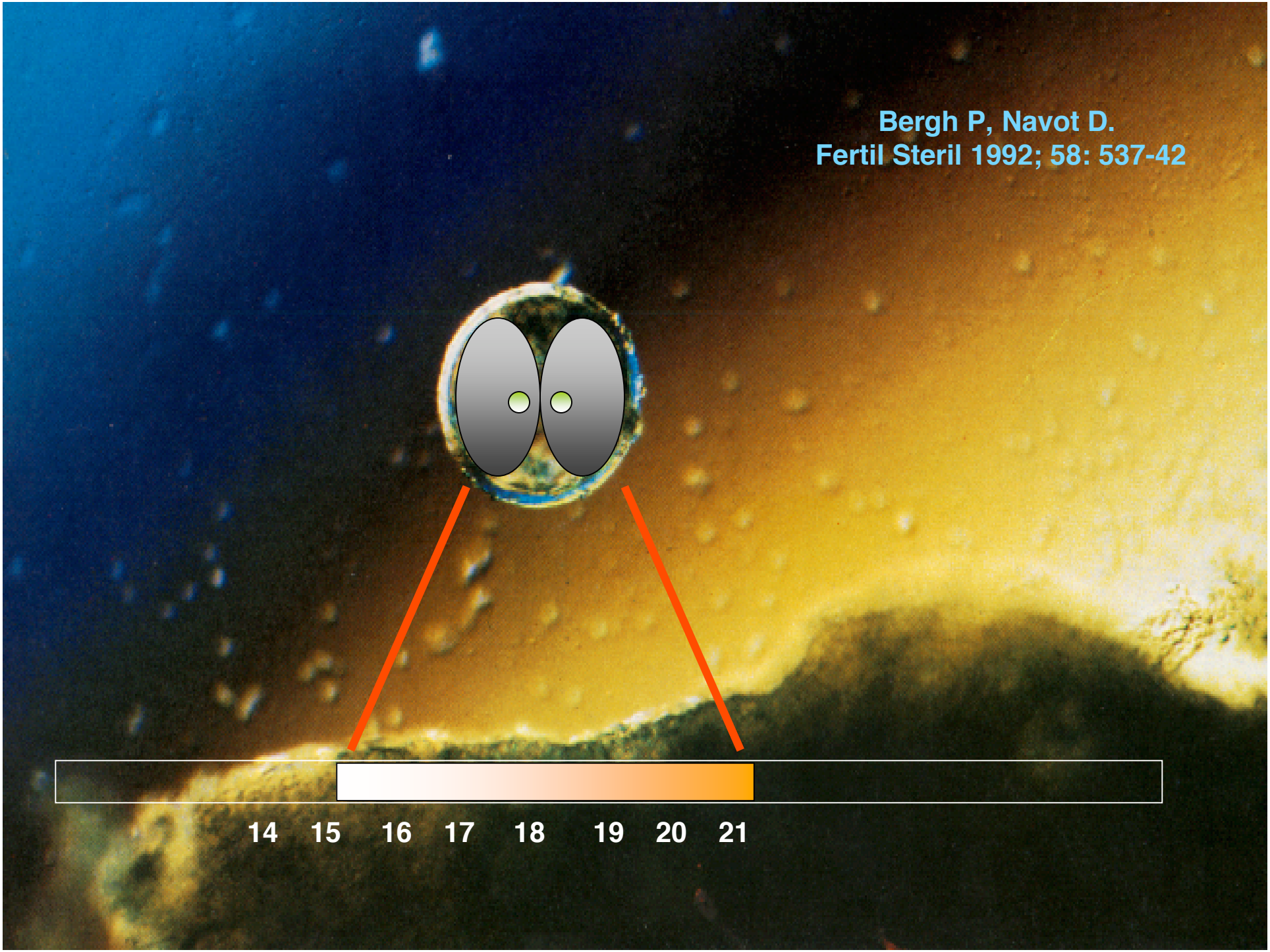
Players involved in implantation

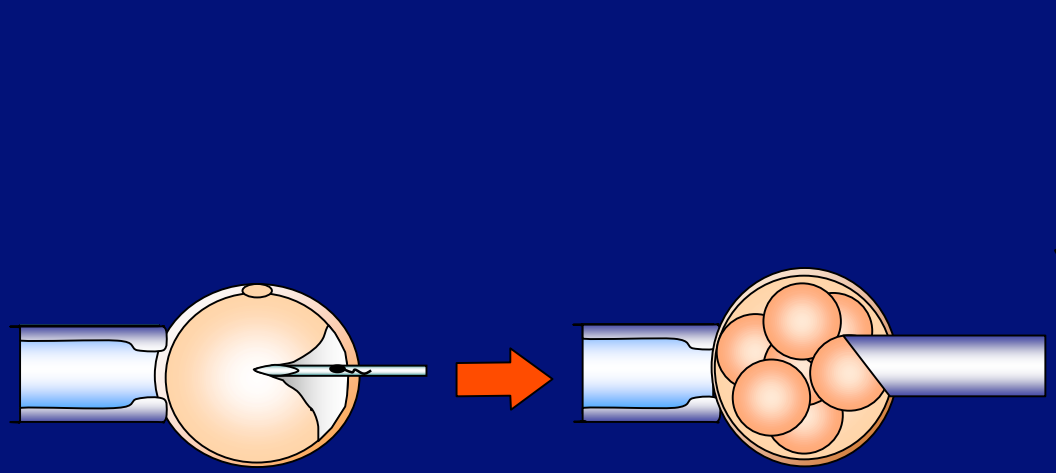


Embryo
(morfologic & genetic)

Endometrium
(morfologic & genetic)


Bergh P, Navot D.
Fertil Steril 1992; 58: 537-42
14 15 16 17 18 19 20 21

Methodology



ICSI

(day 0)

EMBRYO BIOPSY

(day 3)

COCULTURE/ TRANSFER

(day 5)

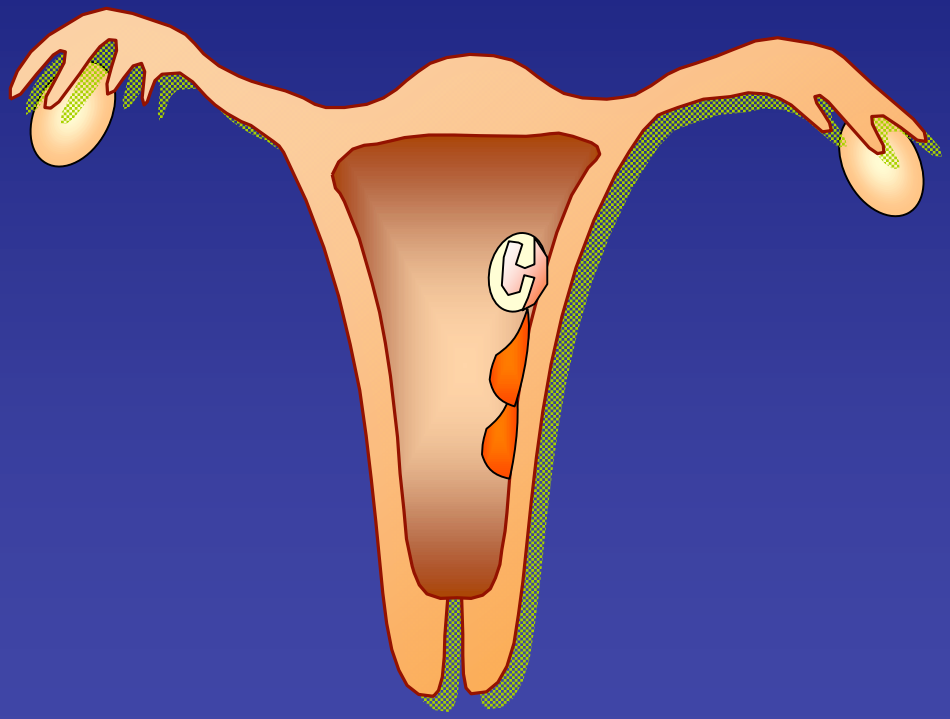
FISH

Biochemical pregnancies in chromosomally normal embryos

	PGD	Day 3	Blastocyst	p value
Cycles	62	62	62	
Age [a]	34.4 ± 3.7 [b]	33.4±3.6	32.7 ±3.5 [b]	0.034
Days of treatment	10.7 ± 1.9	10.2±1.8	10.3 ±1.6	0.409
Gonadotropins [a]	3967 ± 1932 [c]	3613±1854	2588 ± 985 [c]	0.02
E2 day hCG [a]	3586 ±1621 [d]	2592±1363 [d]	3458 ±1365	0.01
Oocytes [a]	21.3 ± 11.2	16.4±8.6 [e]	25.4 ±10.7	<0.001
Embryos transfer [a]	2.1 ± 0.9 [f]	2.9±0.8	2.7 ± 0.7	<0.001
Implantation rate [a]	51.5 ± 38.8	50.2±34.2	47.3 ±36.4	0.8
Biochemical (%)	16 (25,8)	15 (24,2)	16 (25,8)	>0.05

Troncoso et al, Fertil Steril 2003; 79: 449

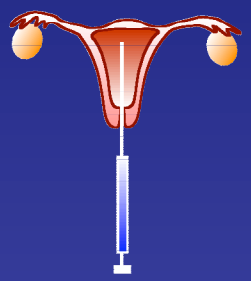
When implantation fails?



PATIENTS

urine samples

0 6 8 10 12 14 16



MEIA (Abbott diagn. Madrid. Spain)

Sensitivity = 2mUI/ml

IVF n = 145

Control group: Oocyte donors n = 15

Oocyte donation n = 92

Control group: Mock cycle n = 10

Test pack + plus

sensitivity >25 mUI/ml

Simón et al. Fertil Steril 1999; 72: 1061

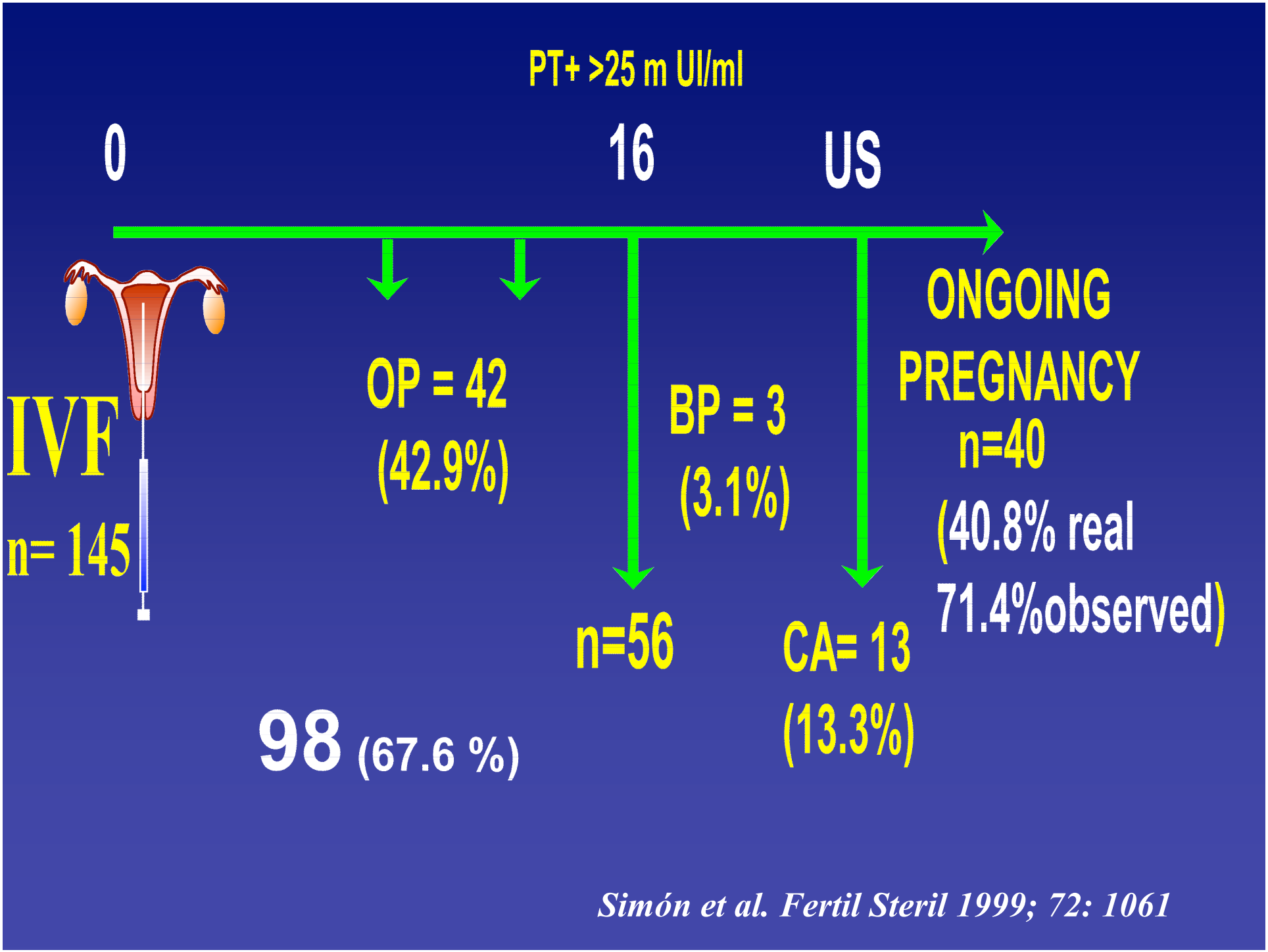

PT+ >25 m UI/ml
0
16
US
IVF
n= 145
OP = 42
(42.9%)
BP = 3
(3.1%)
ONGOING
PREGNANCY
n=40
(40.8% real
71.4%observed)
n=56
CA= 13
(13.3%)
98 (67.6 %)
Simón et al. Fertil Steril 1999; 72: 1061

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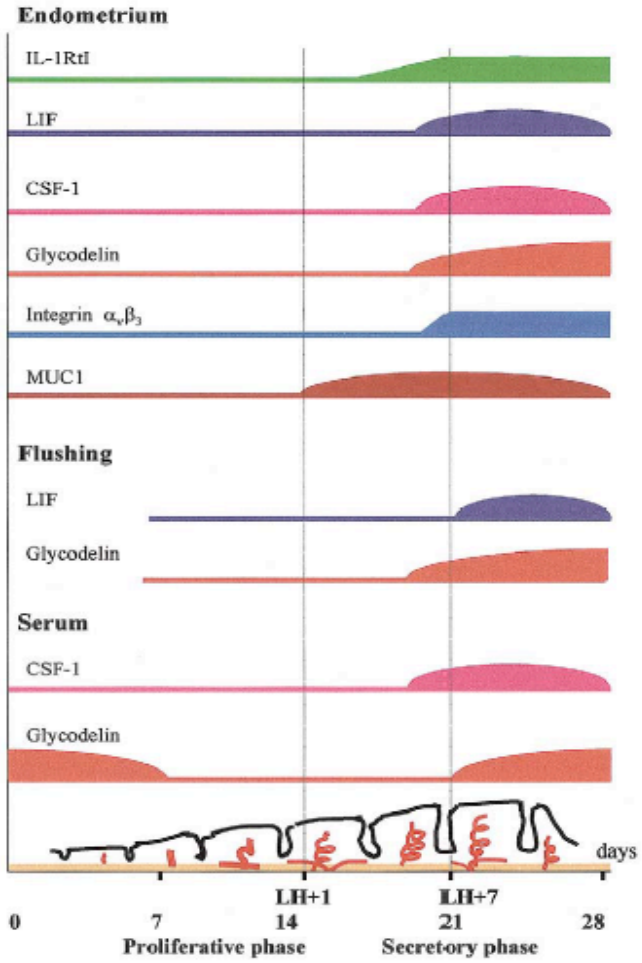
FIGURE 1

Profiles across the menstrual cycle of factors of importance to the interaction between human blastocyst and endometrium: interleukin 1 receptor type 1 (IL-1Rt1), leukemia inhibitory factor (LIF), colony-stimulating factor-1 (CSF-1), glycodelin, integrin $\alpha_v\beta_3$, and MUC1.



Lindhard. Endometrial function at implantation. Fertil Steril 2002.

Lindhard et al, Fertil Steril 2002; 78: 221

High within-subject between cycle variability in markers of endometrial receptive status

Table 2. Cycle to cycle agreement for each endometrial variable investigated in three consecutive cycles in 15 patients.

Variable	1st biopsy		Agreement between 1st and 2nd biopsies			Agreement between 1st and 3rd biopsies		
	Yes	No	%	κ-value (P)	Level of agreement	%	κ-value (P)	Level of agreement
Histological dating within the implantation window	8 (53%)	7 (47%)	66.7	0.32 (0.10)	Fair	60.0	0.21 (0.19)	Fair
Biopsy in phase	12(80%)	3 (20%)	66.7	-0.19 (0.78)	Poor	60.0	0.00 (0.50)	Slight
αvβ3 expression	6 (40%)	9 (60%)	33.3	-0.39 (0.93)	Poor	60.0	0.21 (0.19)	Fair
Pinopod formation	12(80%)	3 (20%)	60.0	-0.25 (0.83)	Poor	73.3	-0.11 (0.70)	Poor

Ordi et al. J Clin Endocrinol Metab 2003; 88:2119

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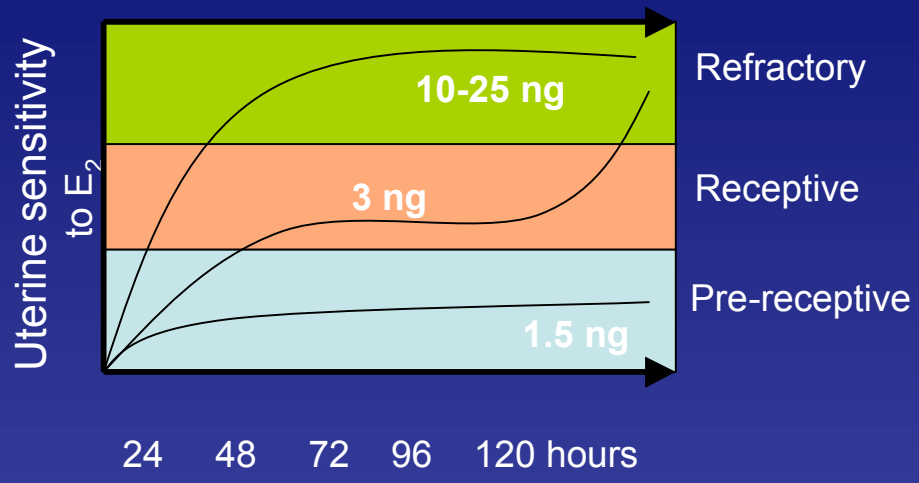
Clinical implications

- High responders have lower implantation rates compared to normal responders due to alteration of endometrial receptivity (***Simón et al, Hum Reprod 1995. Valbuena et al, Hum Reprod 1999***)

- Determination of peri-implantation estradiol levels (E_2) in high versus normal responders (***Pellicer et al, Fertil Steril 1996. Valbuena et al, Hum Reprod 1999***)

- Increasing implantation rates by decreasing estradiol levels during the preimplantation period in high responders with the use of a step-down regimen ***(Simón et al, Fertil Steril 1998. Valbuena et al, Hum Reprod 1999)***



Genes associated with uterine preparation to the receptive state in mice (COX-1, Lif, Hoxa 10, amphiregulin) are aberrantly expressed after high dose of estrogen

Ma et al. PNAS 2003; 100: 2963-8

LEUKEMIA INHIBITING FACTOR (LIF)

- *Concentration of LIF in uterine flushing is predictive of embryo implantation*

  (Lédée-Bataille et al, Hum Reprod 2002;17: 213)

- *Administration of LIF improves implantation*

  (ESHRE, 2003, 0-058)

- *Prospective Multicenter study*

CONCLUSIONS

- *There are endometrial-embryonic interactions at the molecular level, but the embryo seems to drive all these orchestrated events*
- *The endometrium is an independent and important factor in implantation*
- *The screening methods of endometrial receptivity are difficult to apply due to individual variability and effect of the embryo*
- *IVF outcome can be improved through enhanced endometrial receptivity*

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Ana Cobo
J.A. G. Velasco
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Wen-ge, M. *et al.* *Proc.Natl. Acad. Sci. U. S. A.* (2003)