

IMPACT OF OVARIAN STIMULATION ON THE LUTEAL ENDOMETRIUM

Professor IOANNIS E. MESSINIS
MD, PhD (Aberdeen, UK), FRCOG (UK)

Department of Obs/Gynae
University of Thessaly
Larissa, Greece



DISCLOSURE

Nothing to disclose

Learning Objectives

At the conclusion of this presentation, the participant should be able to:

- Discuss changes in hormone secretion in stimulated cycles**
- Describe the impact of high levels of ovarian steroids on endometrial maturation**
- Summarize morphological and functional genomic changes in the endometrium**
- Evaluate the impact of changes in endometrial receptivity on clinical outcome**

OVARIAN STIMULATION

(Luteal phase)

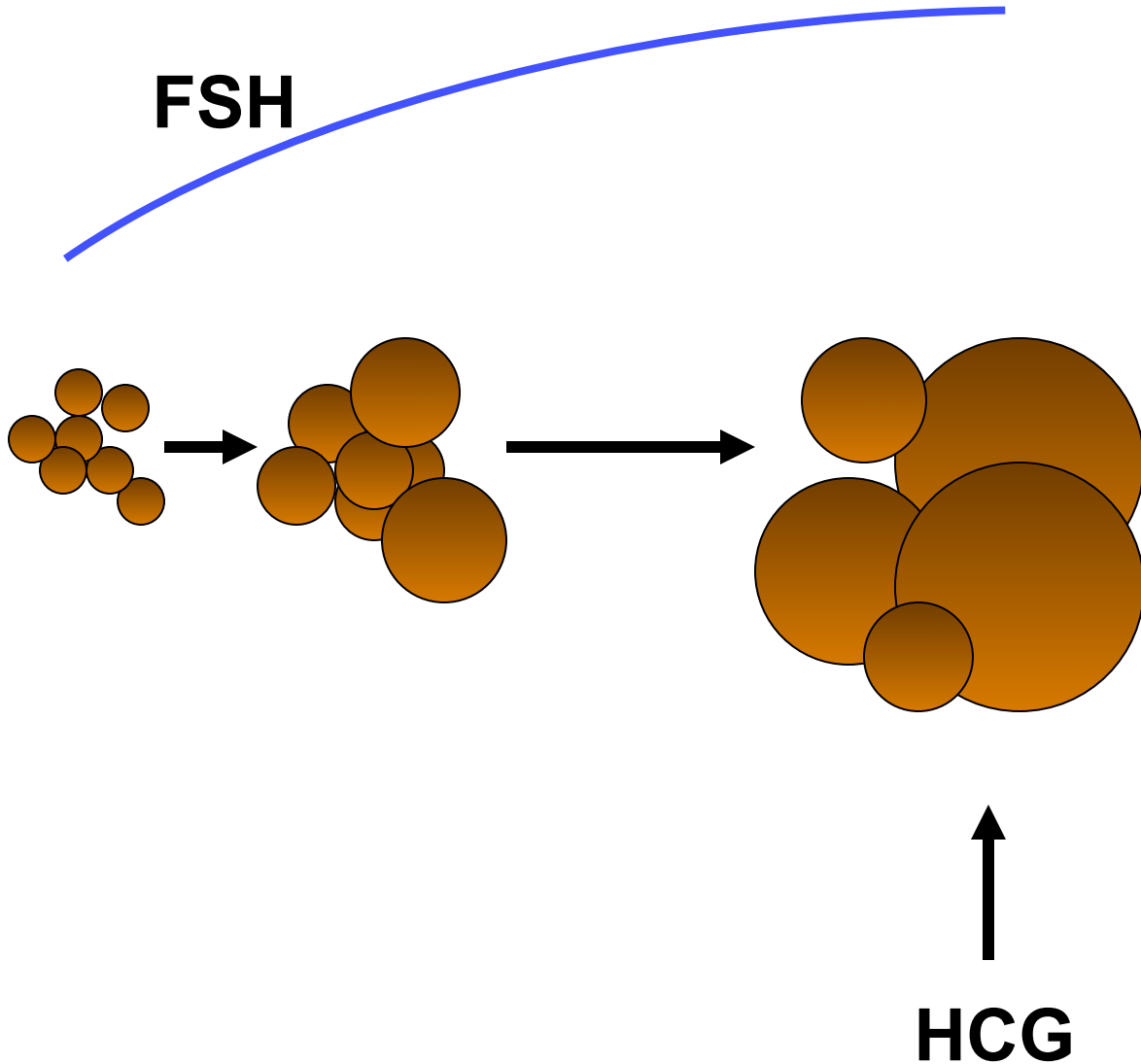
- **Endocrine changes**
- **Endometrial morphology changes**
- **Gene transcripts**
- **Clinical outcome**
- **Luteal support**

OVARIAN STIMULATION

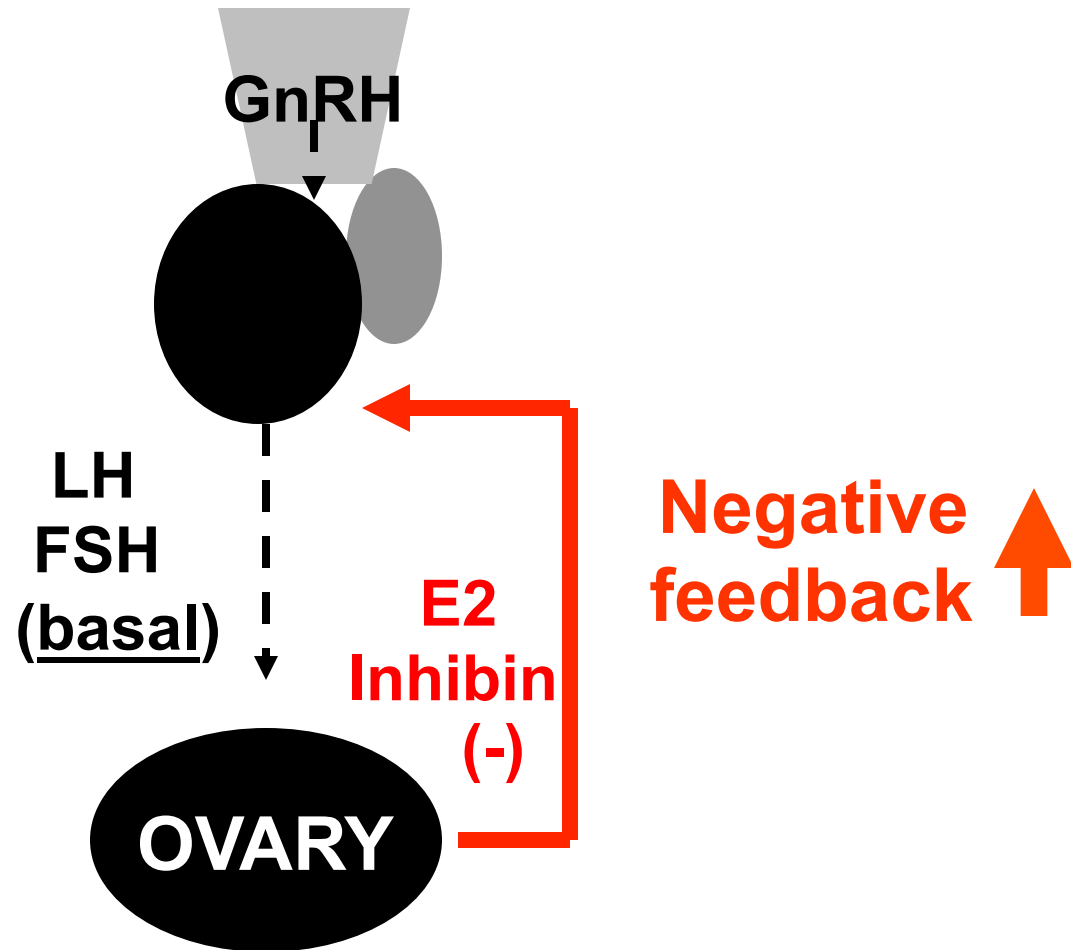
(Luteal phase)

- **Endocrine changes**
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MULTIPLE FOLLICLES



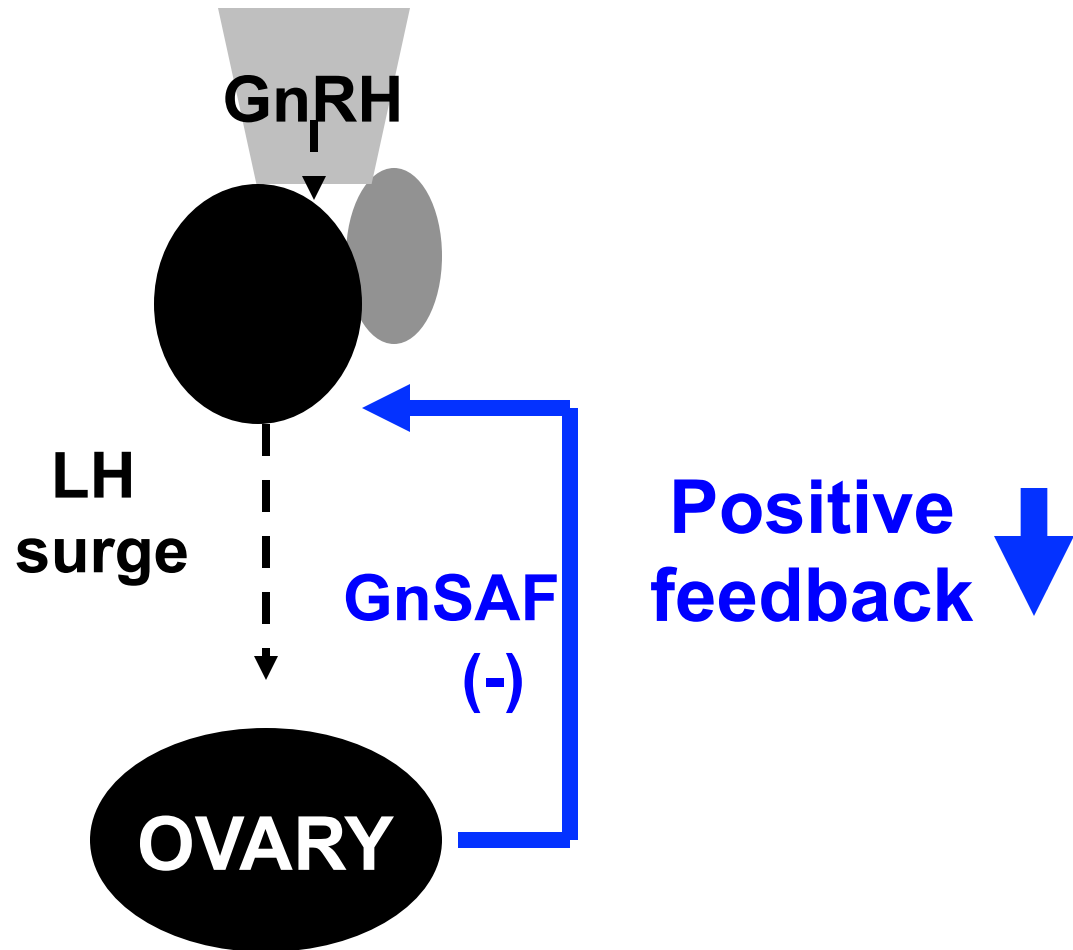
POTENTIATION OF THE NEGATIVE FEEDBACK



Multiple follicular development

Messinis, 2006
Hum. Reprod. Update 12, 557-571

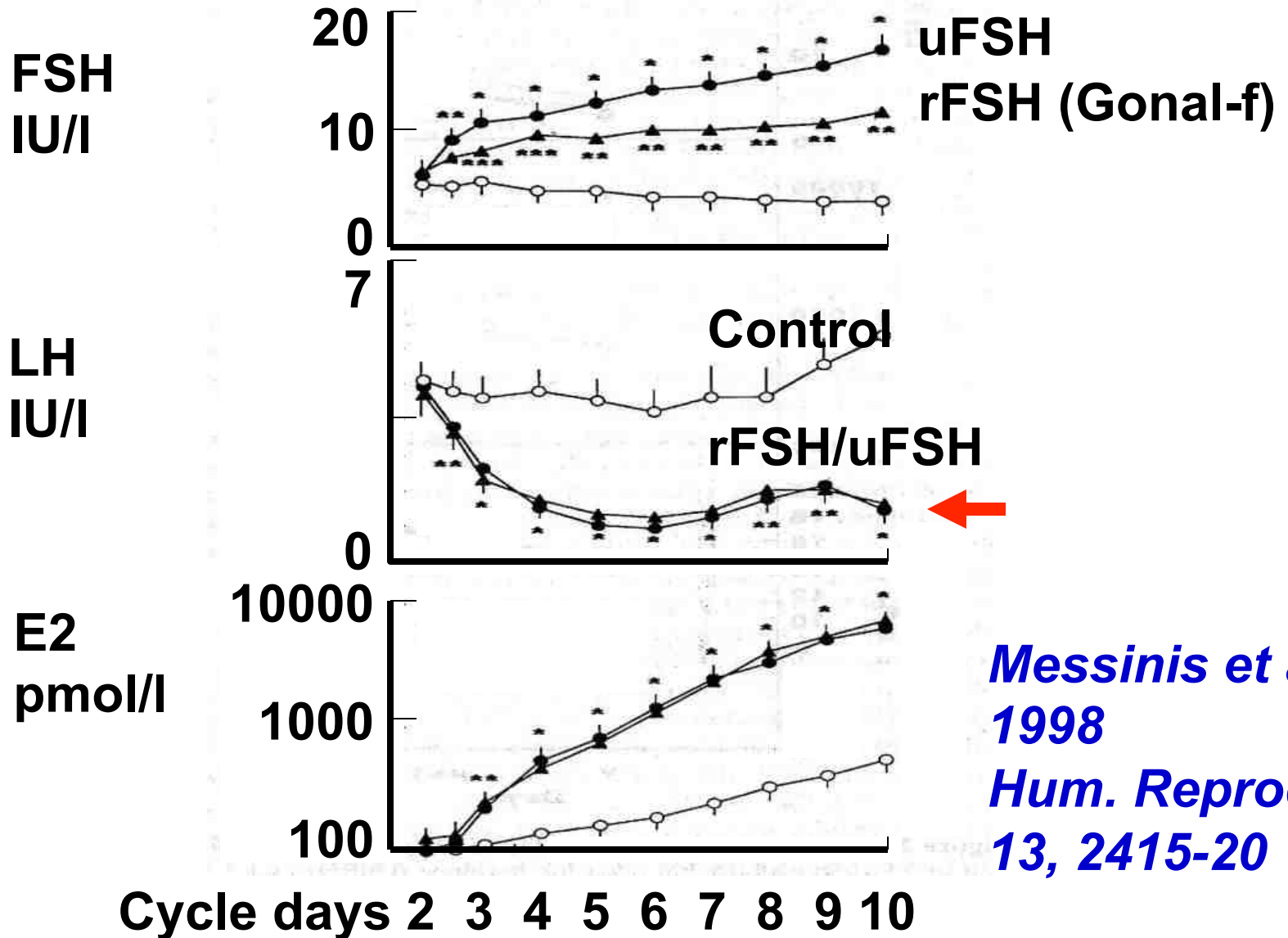
ATTENUATION OF THE POSITIVE FEEDBACK



Multiple follicular development

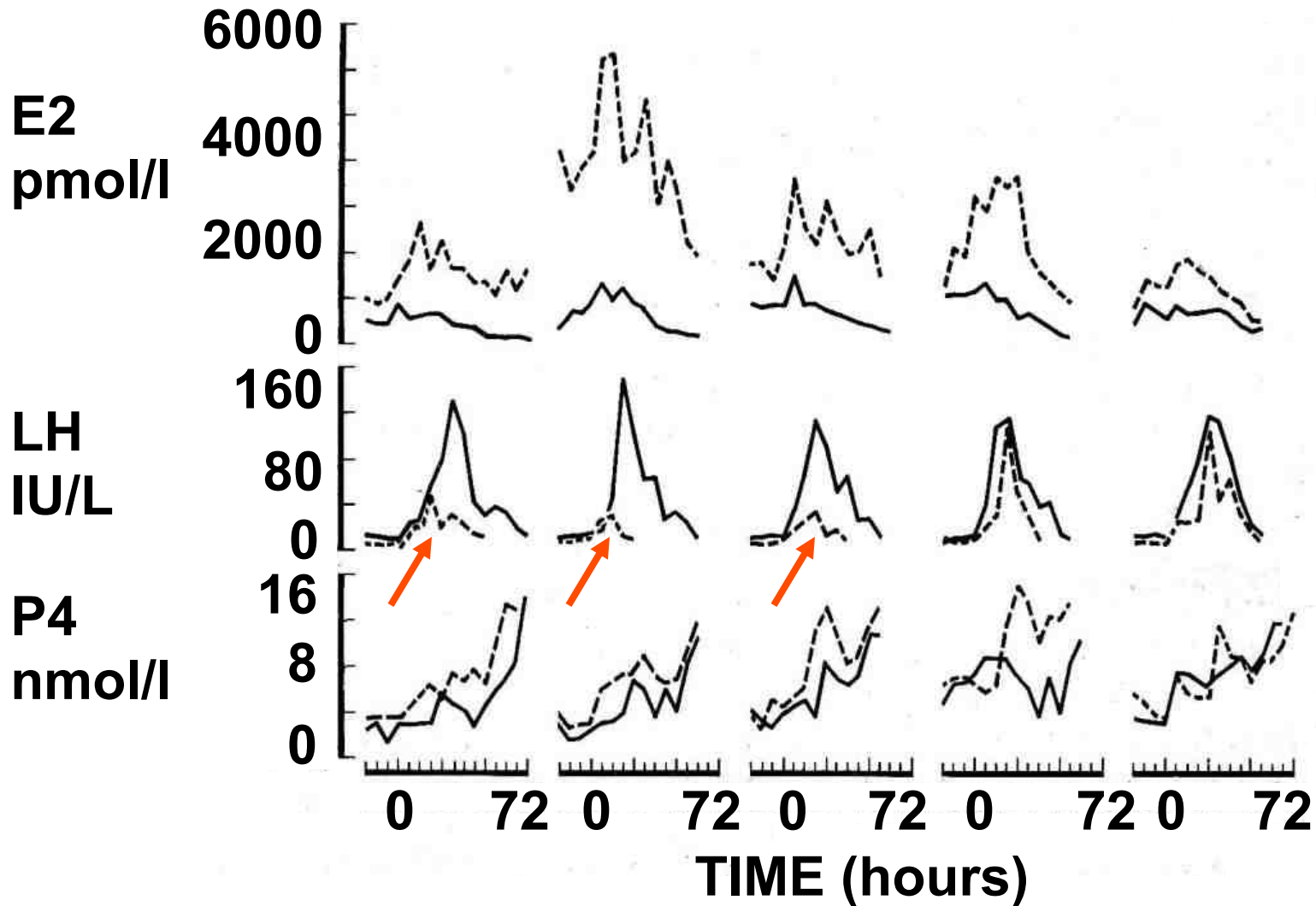
*Messinis, 2006
Hum. Reprod. Update 12, 557-571*

LH IS SUPPRESSED



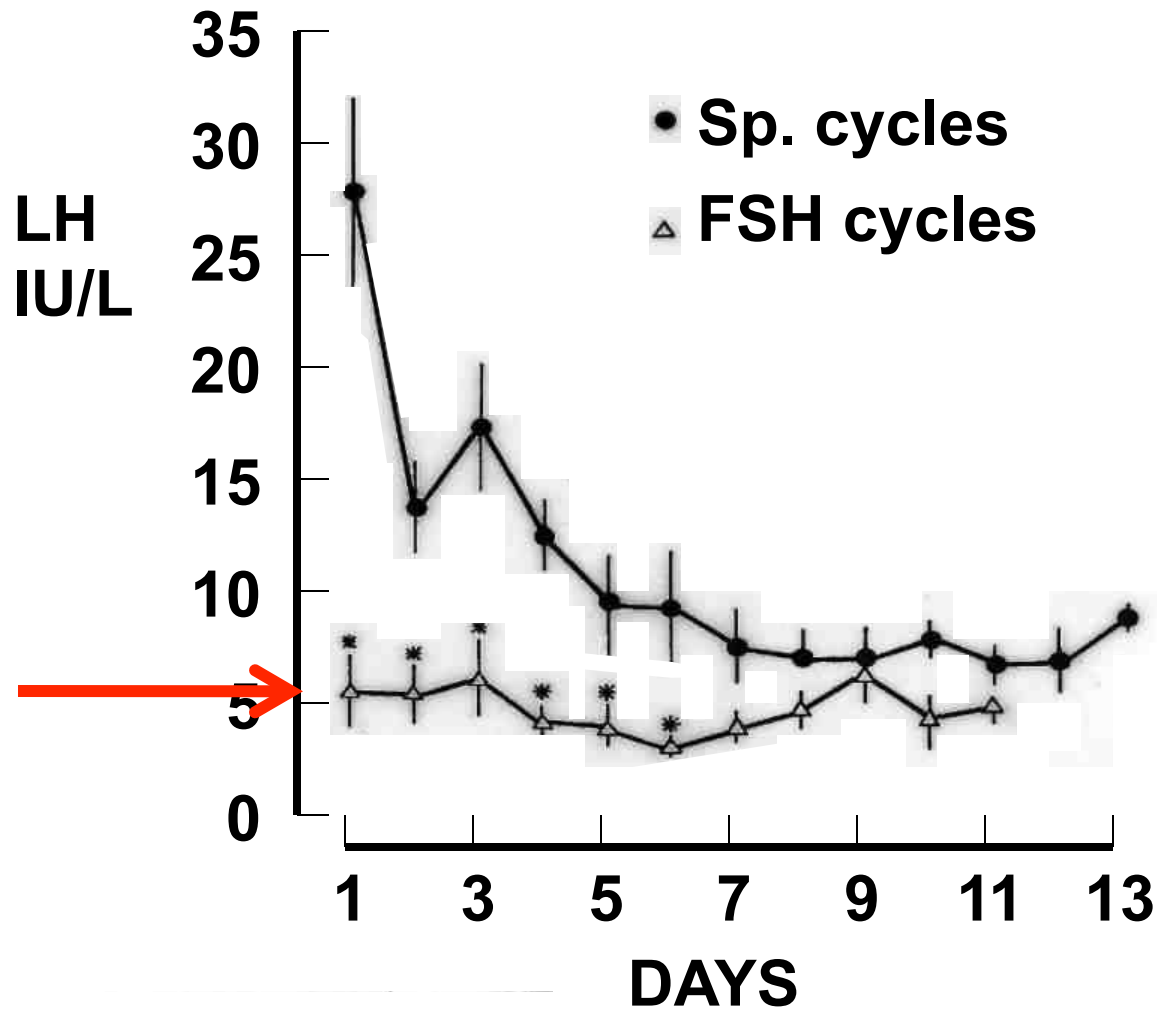
*Messinis et al.,
1998
Hum. Reprod.
13, 2415-20*

SUPEROVULATION INDUCTION WITH PULSATILE FSH (Attenuated LH surges)



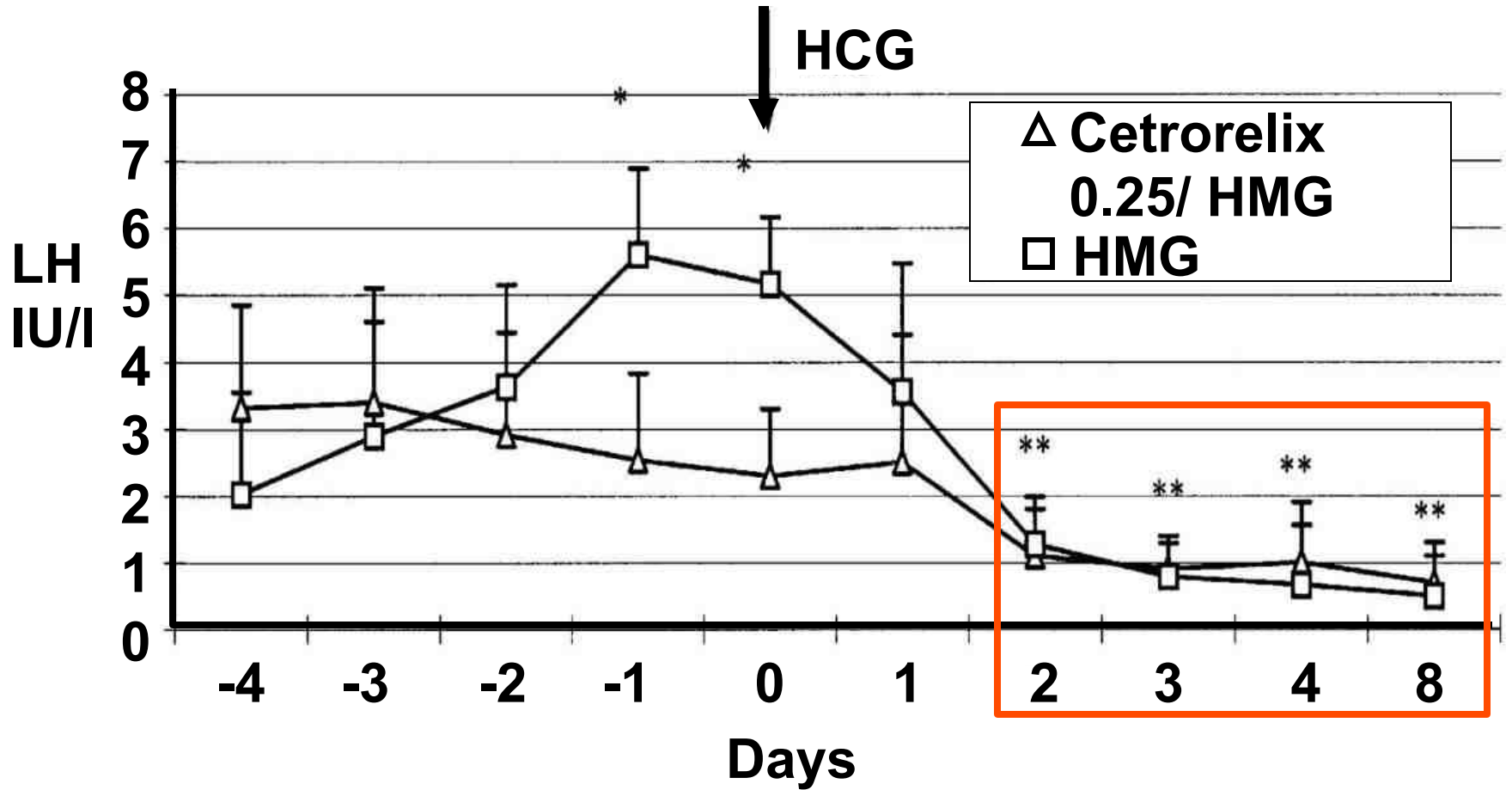
Messinis & Templeton, 1986; Clin. Endocrinol. 25, 633-640

LUTEAL PHASE LH VALUES FOLLOWING AN ATTENUATED LH SURGE



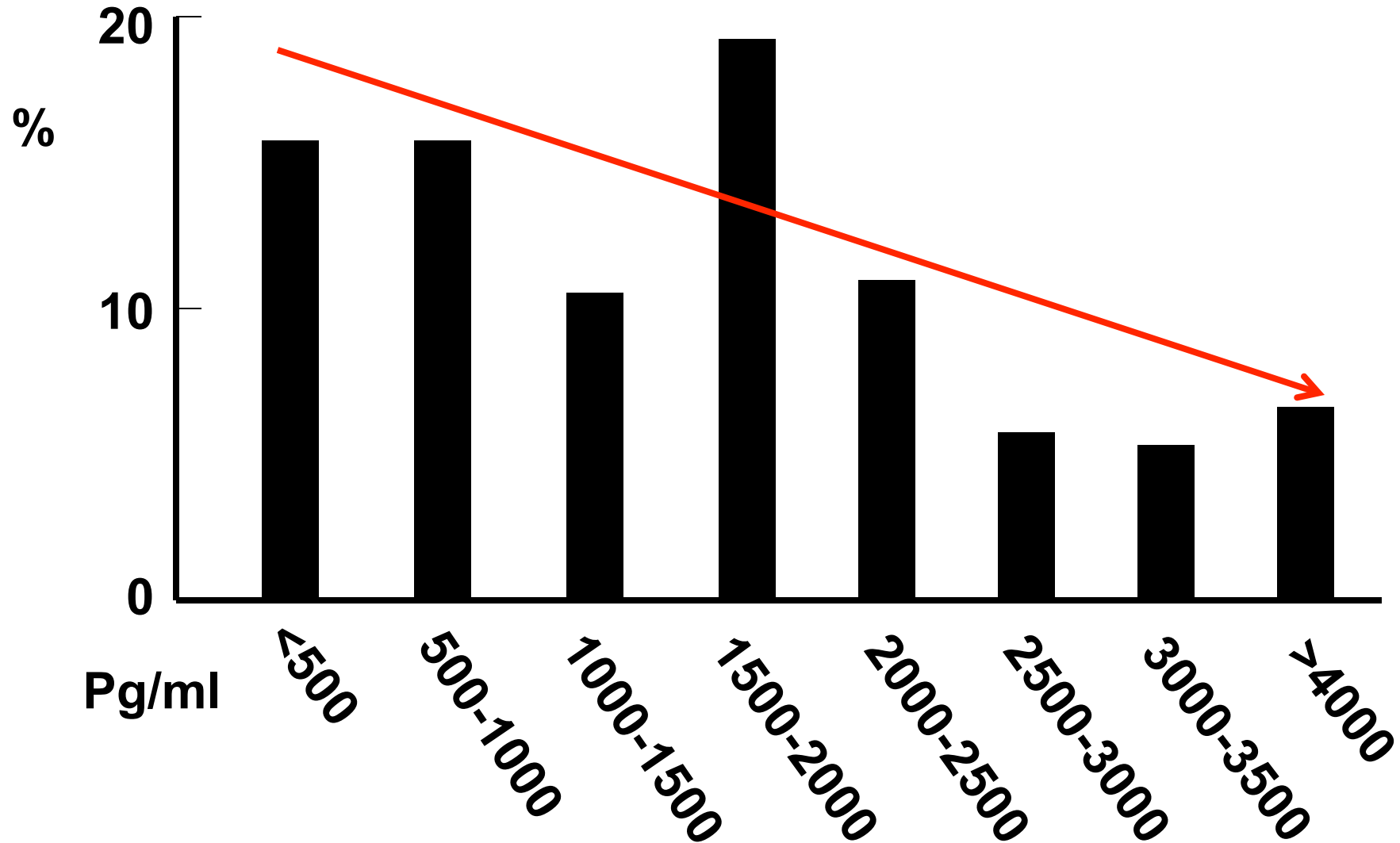
*Messinis & Templeton, 1987
J. Reprod. Fert. 79, 549-554*

SERUM LH VALUES (Luteal)



*Tavaniotou et al., 2001
Hum. Reprod. 16, 663-667*

IMPLANTATION RATE AND ESTRADIOL LEVELS ON DAY OF HCG



Simon et al., 1995; Hum. Reprod. 10, 2432-7

SERUM PROGESTERONE

CYCLES	DAY OF HCG INJECTION	DAY OF FOLLICLE ASPIRATION
--------	----------------------------	----------------------------------

Unstimulated

0.5±0.2

0.5±0.1

Hyperstimulated

1.1±0.6*

8.5±2.2**

* P<0.01, ** P<0.001

Kolb & Paulson, 1997

Am. J. Obstet. Gynecol. 176, 1262-7

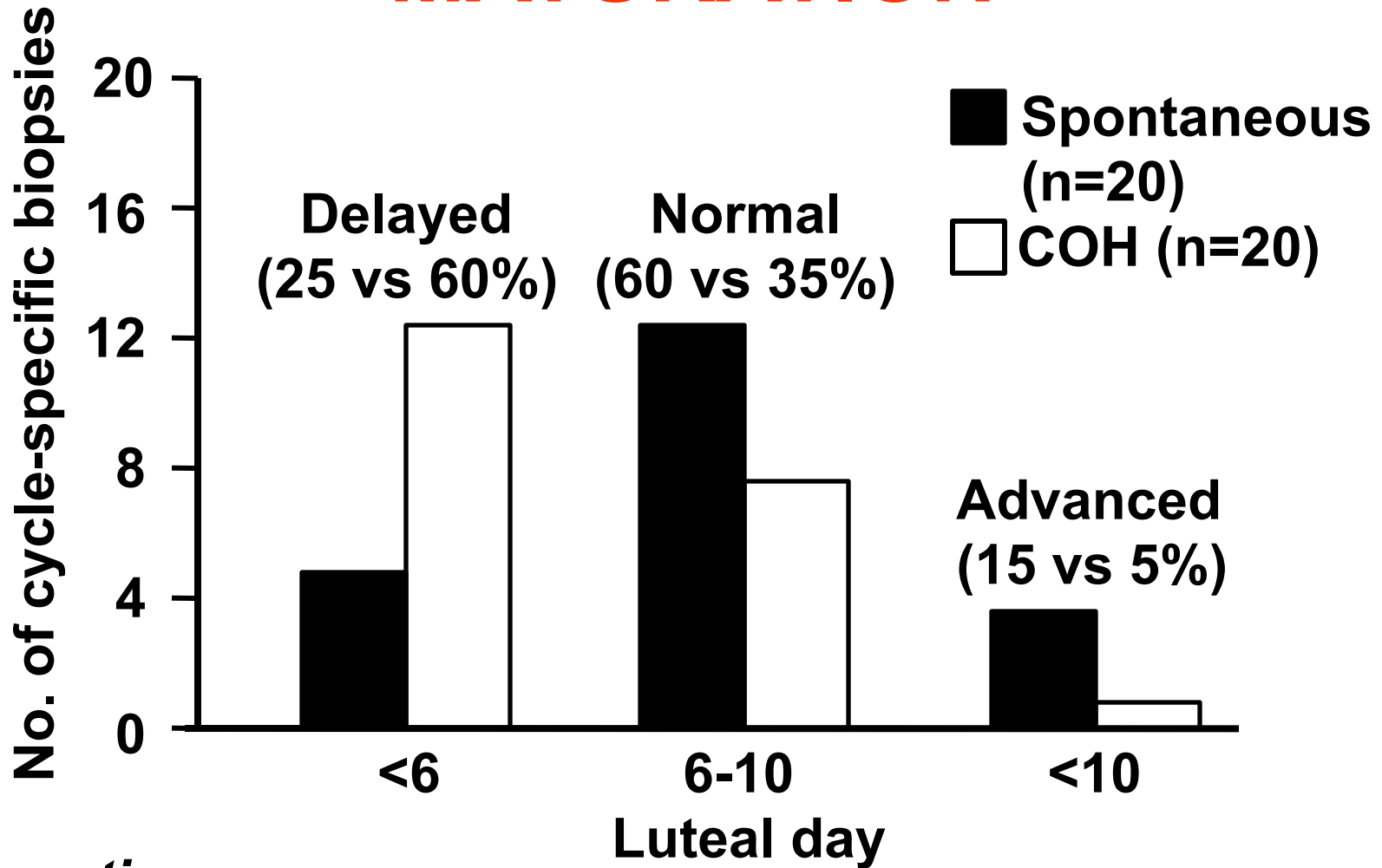
OVARIAN STIMULATION (Luteal phase)

- Endocrine changes
- **Endometrial morphology changes**
- Gene transcripts
- Clinical outcome
- Luteal support

ENDOMETRIAL DATING

- Endometrial histology is advanced
 - at oocyte pick-up in GnRH agonist or antagonist cycles (*Kolibianakis et al., 2003; Saadat et al., 2004*).
 - at mid-luteal phase in 60% of patients (*Kolibianakis et al., 2003*), especially with the agonists (*Simon et al., 2005*).

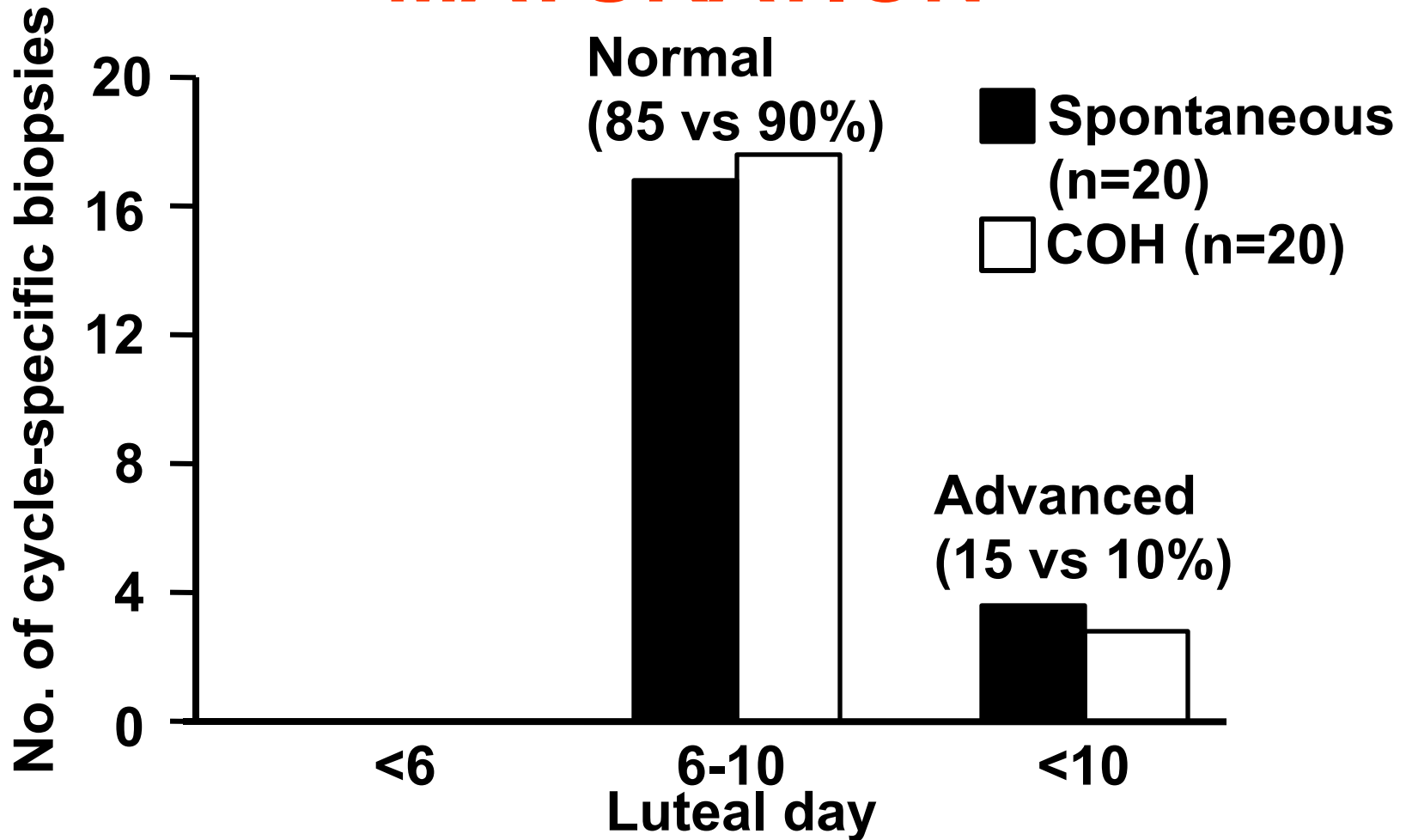
HISTOLOGIC GLANDULAR MATURATION



Prospective
LH/HCG+8 (n=19 oocyte donors) NC
Data not affected by P4 administration

Meyer et al., 1999
Fertil. Steril. 71, 109-14

HISTOLOGIC STROMAL MATURATION



Glandular-stromal dyssynchrony

In 80% of COH

In 30% of spontaneous

Meyer et al., 1999

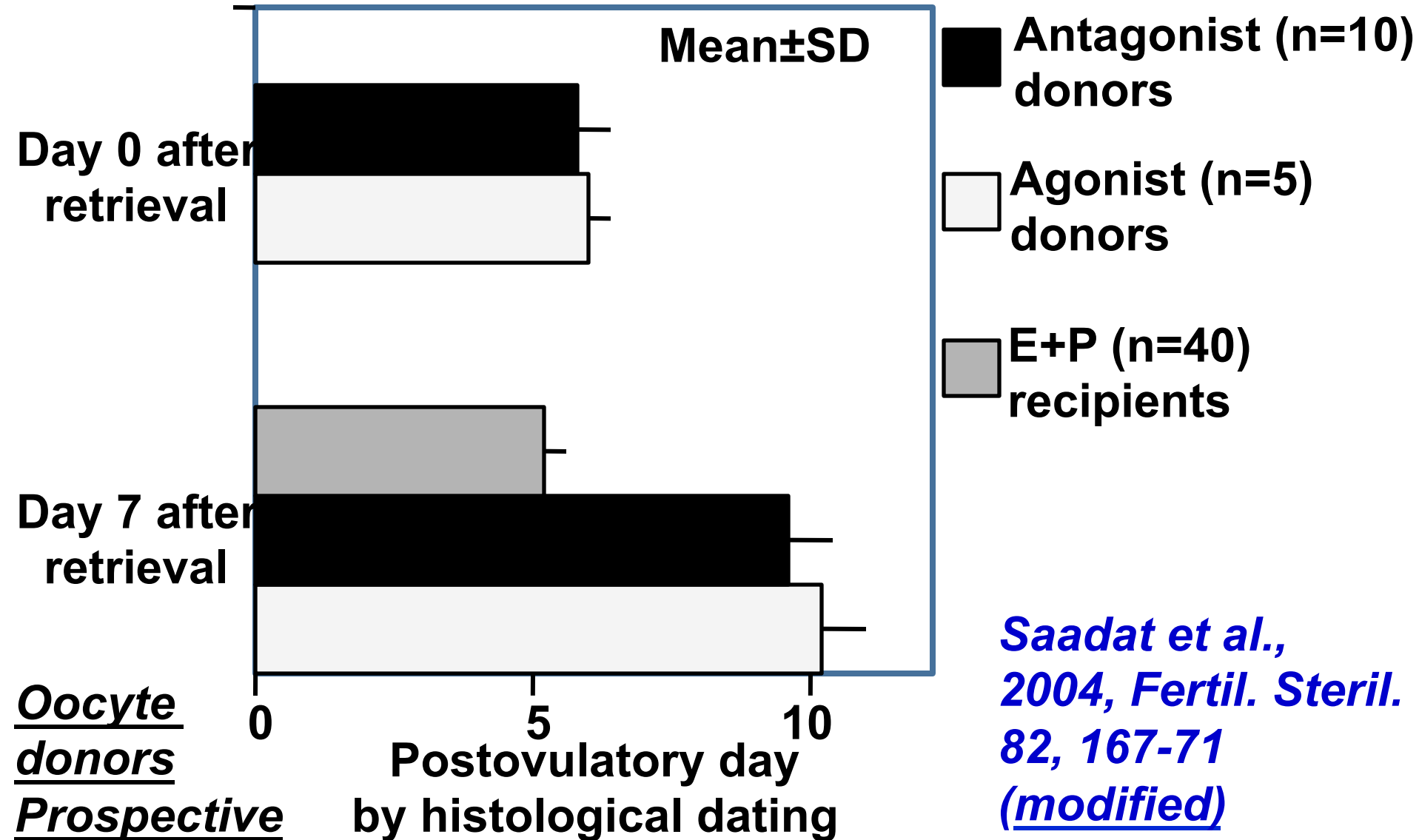
Fertil. Steril. 71, 109-14

THE ENDOMETRIUM IN OVARIAN STIMULATION FOR IVF

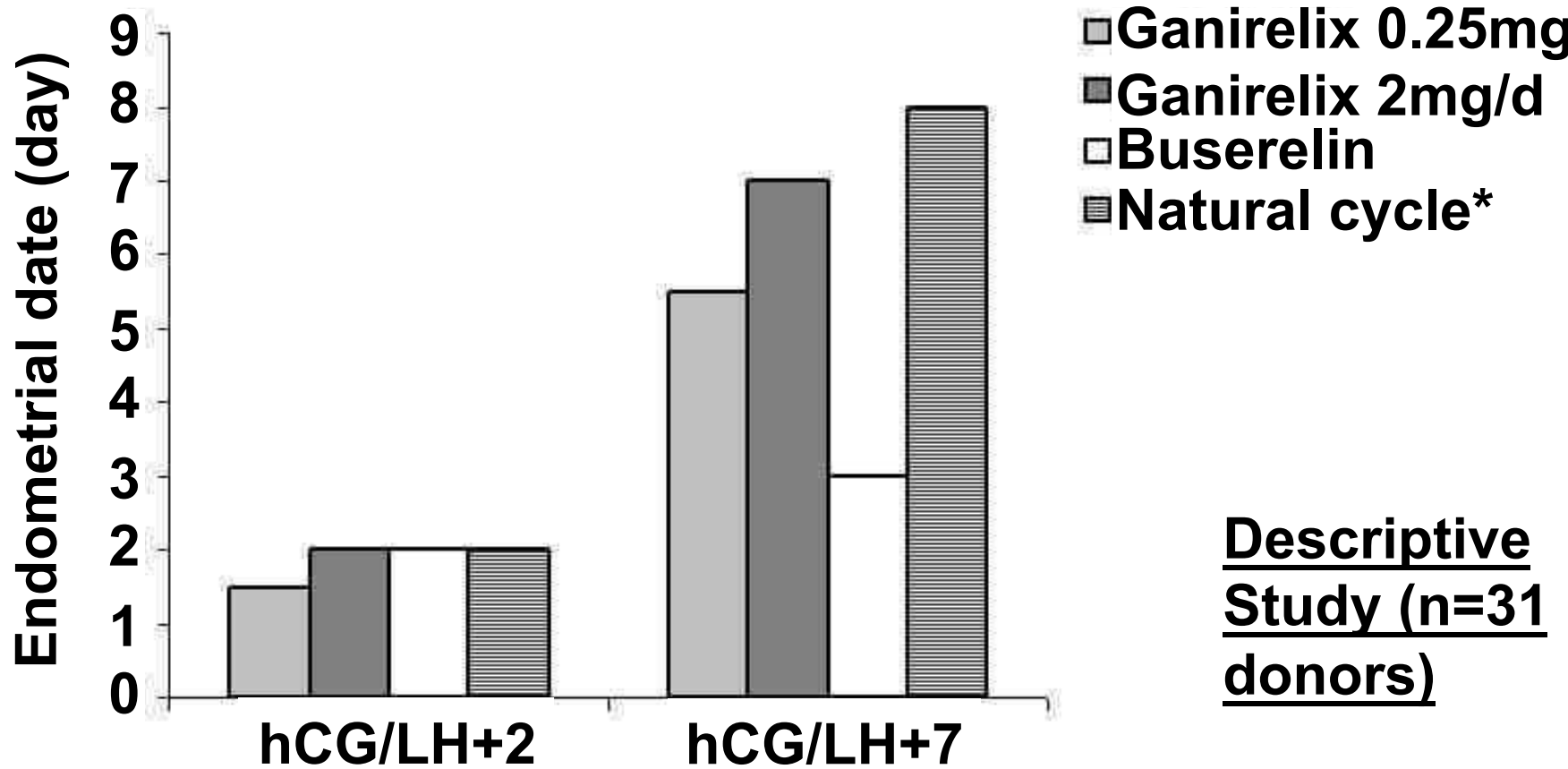
- In peri- and post-ovulatory period → advanced endometrium
- In the early to mid-luteal phase → 'normal' aspect of the endometrium
- In the mid- to late luteal phase → glandular-stromal dyssynchrony

*Bourgain and Devroey, 2003
Hum. Reprod. Update, 9, 515-22*

ACCELERATED ENDOMETRIAL MATURATION



ARRESTED ENDOMETRIUM WITH THE USE OF BUSERELIN



Descriptive
Study (n=31
donors)

* LH peak

Noyes criteria

Simón et al. 2005

Hum. Reprod. 20, 3318-27

L- SE1

EHT- 10.0 KV

WD- 9

mm

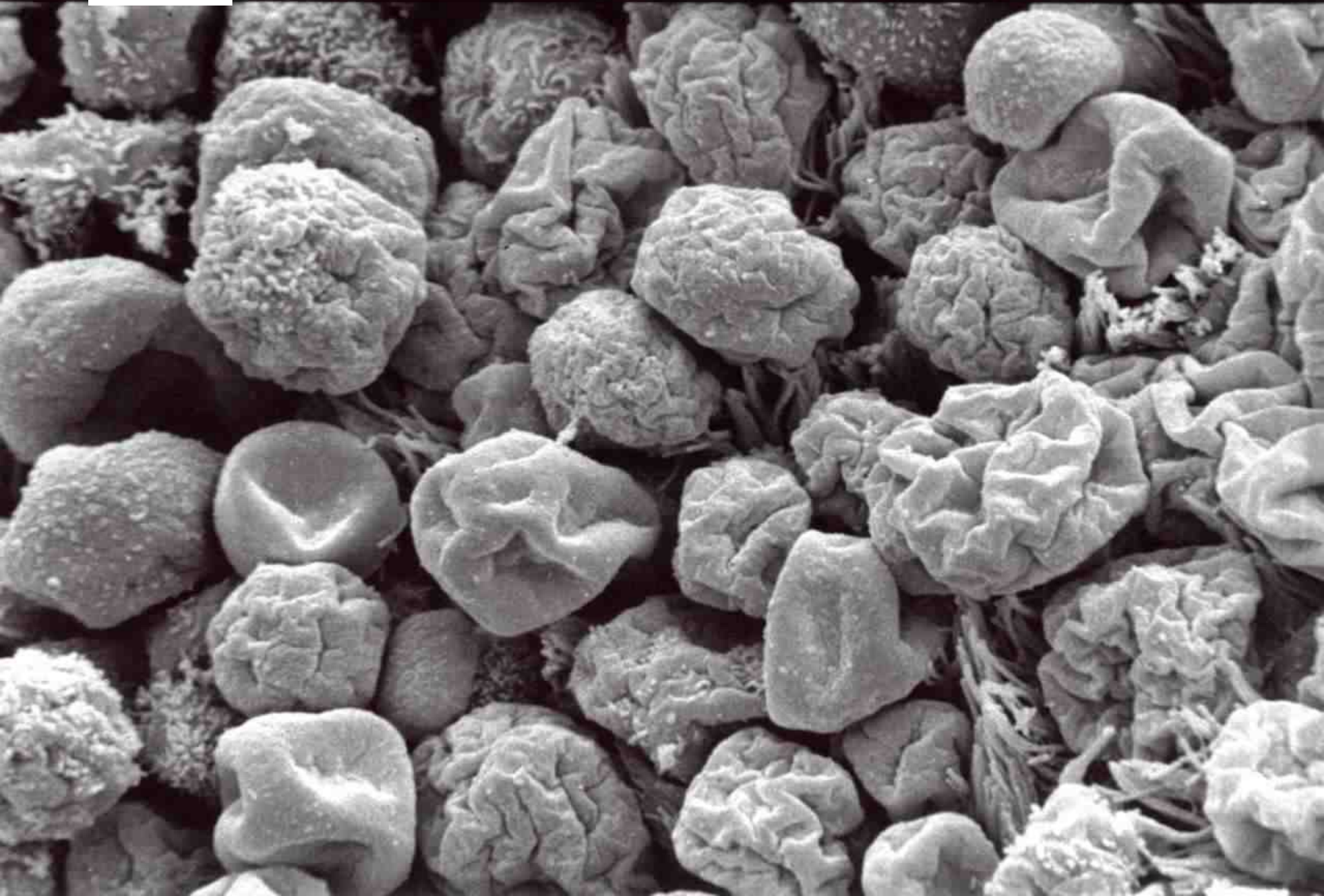
PHOTO- 23937

Day 20

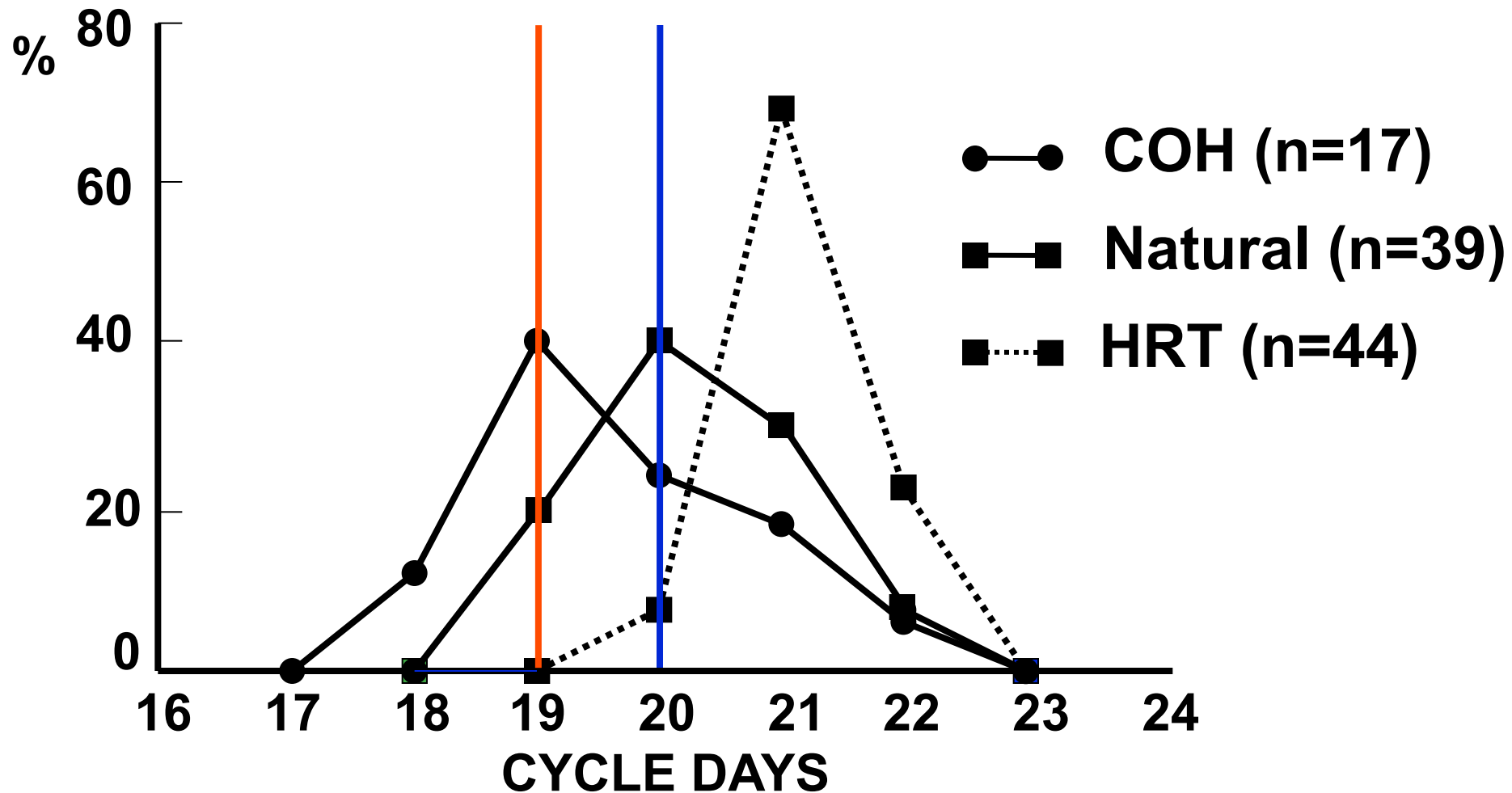
10.0µm



PINOPODES



DIFFERENTIAL EXPRESSION OF PINOPODES



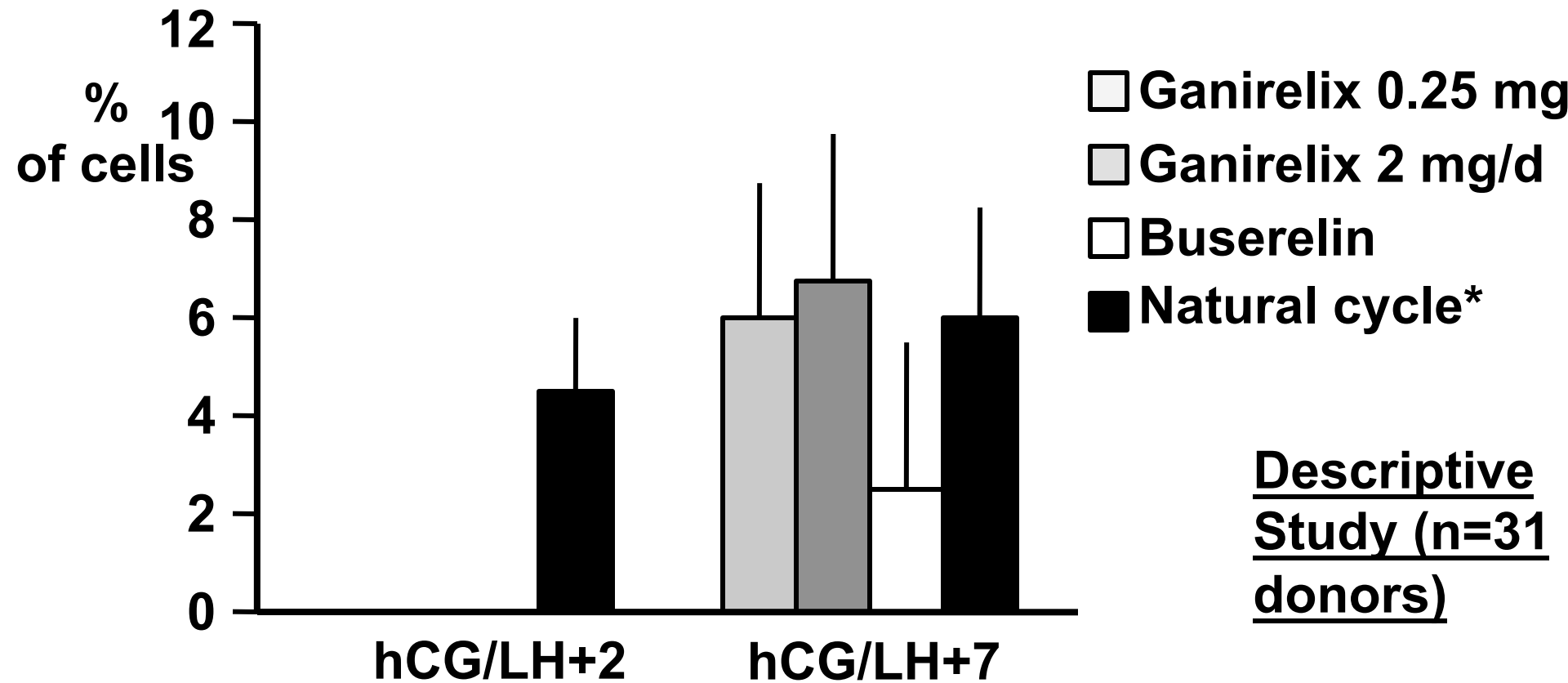
COH CYCLES

(Pinopodes and progesterone receptors)

- Fully developed pinopodes
by day 19 vs day 21 in natural cycles
- The decline in PR staining started
on day 18 vs day 20 in natural cycle

*(Develioglou et al., 1999; Fertil. Steril. 71, 1040-7
Stavreus-evers et al., 2001; Fertil. Steril. 76,
782-91)*

REDUCED EXPRESSION OF PINOPODES BY BUSERELIN



Descriptive
Study (n=31
donors)

* LH peak

Simón et al. 2005

Hum. Reprod. 20, 3318-27

RU486 POSTPONED THE DEVELOPMENT OF PINOPODES

- **In rats:**
 - On Pd6-8 vs day 5 in control
(Sarantis et al., 1988, Hum. Reprod. 3, 251-5)
- **In mice:**
 - on Pd1, pinopodes were reduced
(Huang et al., 2005, Acta Pharmacol. Sin. 26, 212-9)

RU486 AND PINOPODES (Women)

PINOPODES PRESENT (HCG+7)

• DONORS*	
- controls	1 of 4 ^a
- study group (RU486)**	4 of 4
• RECIPIENTS	4 of 4

* COH with HMG/GnRH-a/HCG ^aP<0.05
(endometrium was advanced)

** 2.5 mg the day of OR and 2.5 mg the following day

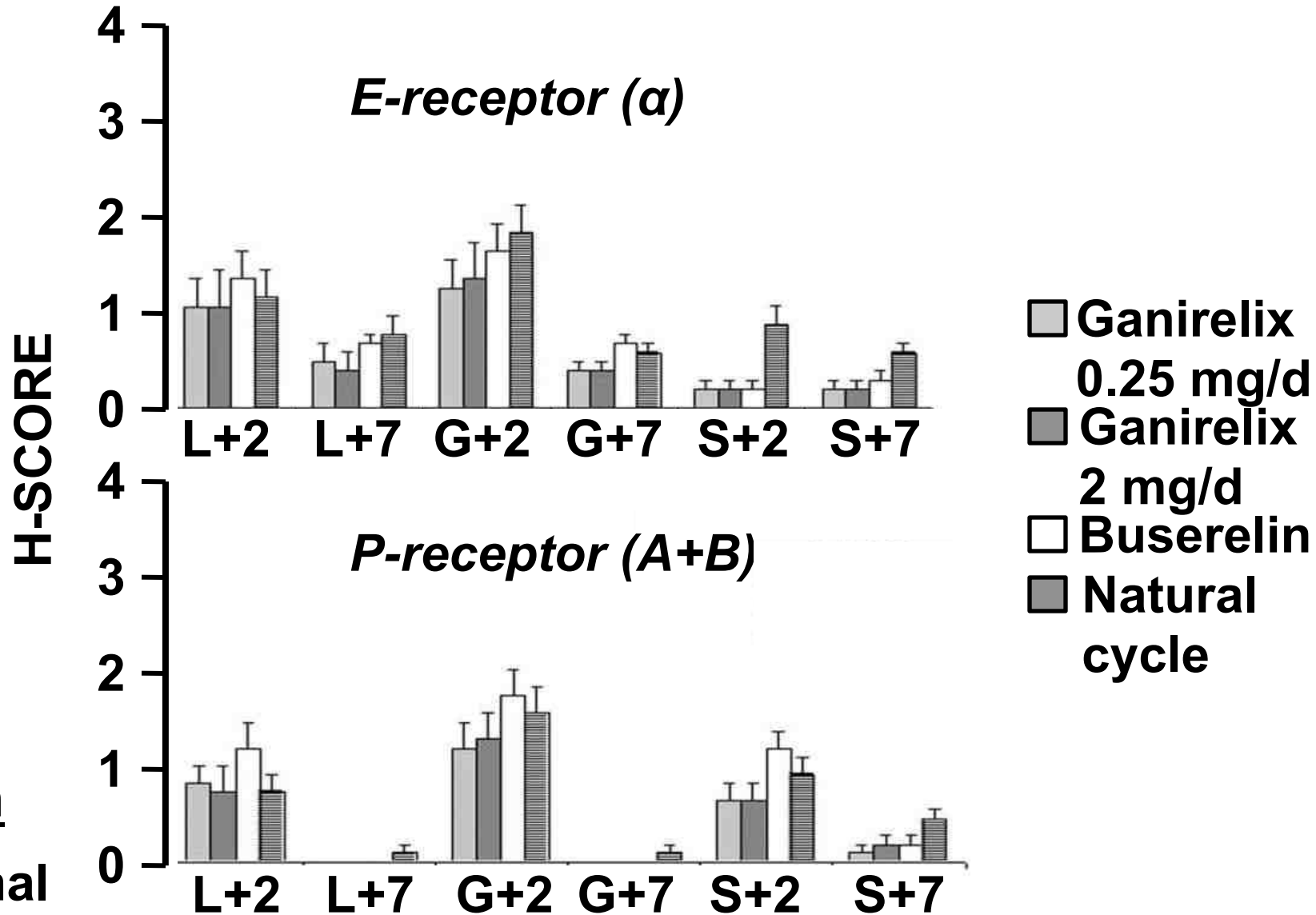
Prosp. CT

Paulson et al., 1997; Fertil. Steril. 67, 321-5

POTENTIAL OF RU486 IN IVF CYCLES

- **To block the endogenous LH surge**
(Messinis et al., 1997; Clin. Endocrinol. 46, 309-14
Escudero et al., 2005; JCEM 90, 2081-8)
- **To postpone the appearance of pinopodes**
(Paulson et al., 1997; Fertil. Steril. 67, 321-5)

RECEPTORS* IN THE ENDOMETRIUM

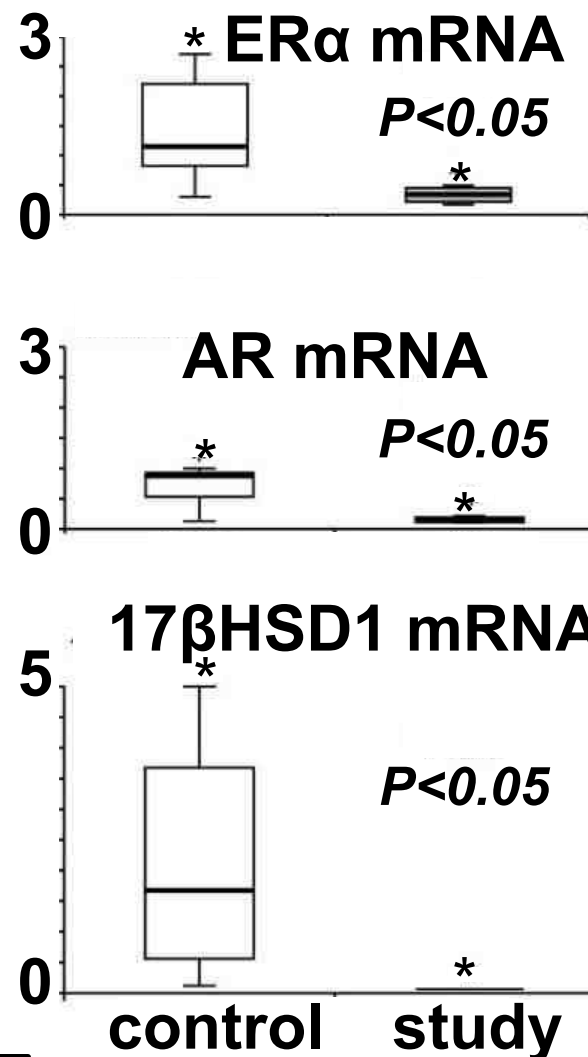
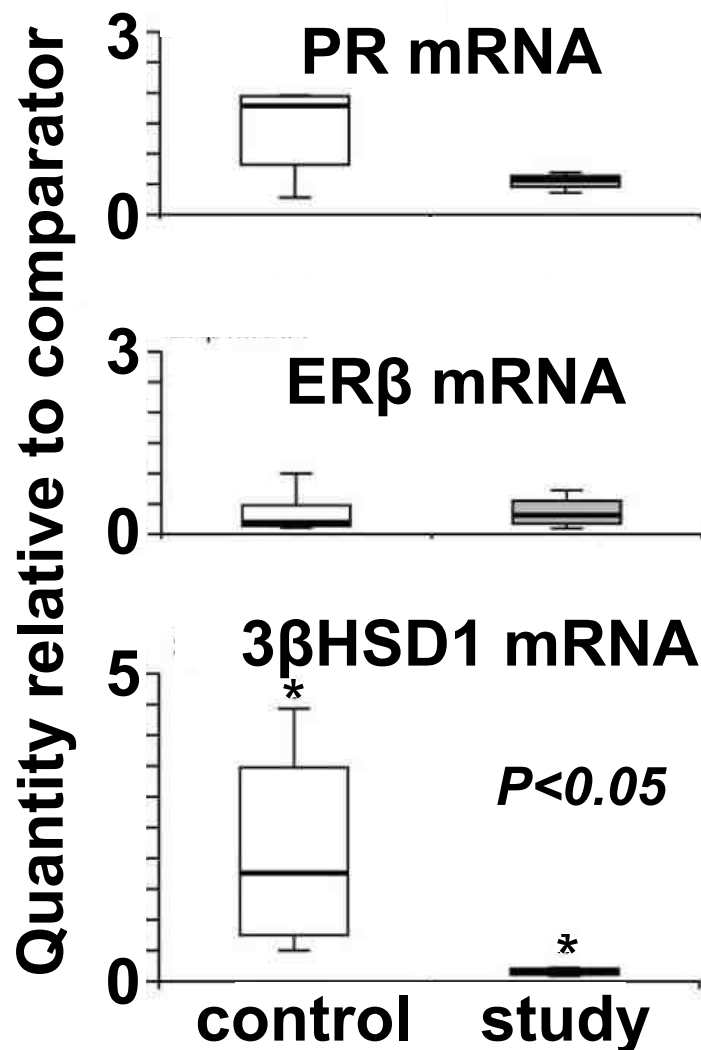


*protein

L=luminal
G=glandular
LH/hCG+2/7

Simón et al. 2005
Hum. Reprod. 20, 3318-27 (modified)

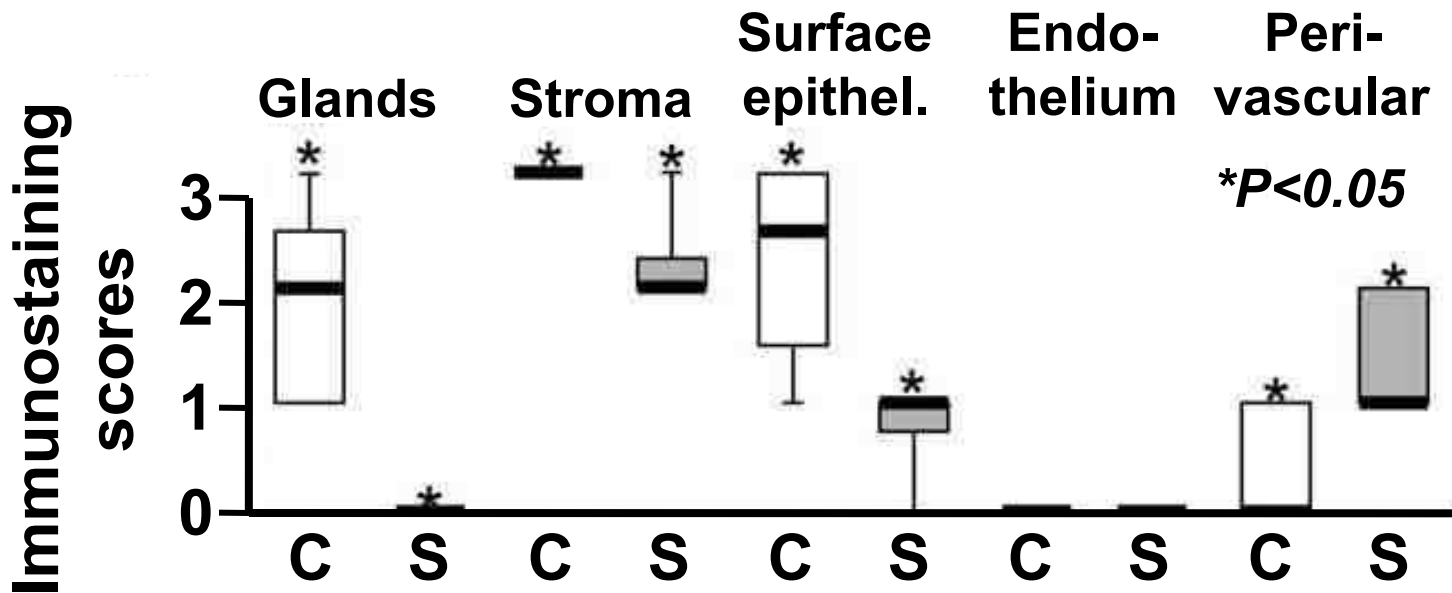
STERIOD RECEPTORS AND METABOLIZING ENZYMES IN MID-LUTEAL ENDOMETRIUM



Donors (study)
COH (rFSH/GnRH Ant+P4 suppl.)

Vani et al., 2007
Hum. Reprod. 22, 2981-91

PROGESTERONE RECEPTOR* IN MID-LUTEAL ENDOMETRIUM



*protein

C: Control group (n=8)

S: Study group (n=5)

Vani et al., 2007

Hum. Reprod. 22, 2981-91

RECEPTORS IN HUMAN ENDOMETRIUM (Immunohistochemistry)

	<u>Natural cycles</u> (n = 12)	<u>Stimulated cycles</u> (n = 23)	P
<u>ER</u>			
<i>Stroma</i>	0.13±0.14	0.17±0.22	NS
<i>Glands</i>	0.38±0.35	0.81±0.53 ↑	.01
<u>PR</u>			
<i>Stroma</i>	1.29±0.23	1.61±0.45 ↑	.02
<i>Glands</i>	2.11±0.43	1.45±0.81 ↓	.02

**LH/HCG+7 (Long agonist)
Infertile, no ET**

***Chai et al., 2011
Fertil. Steril. 96, 764-8***

OVARIAN STIMULATION (Luteal phase)

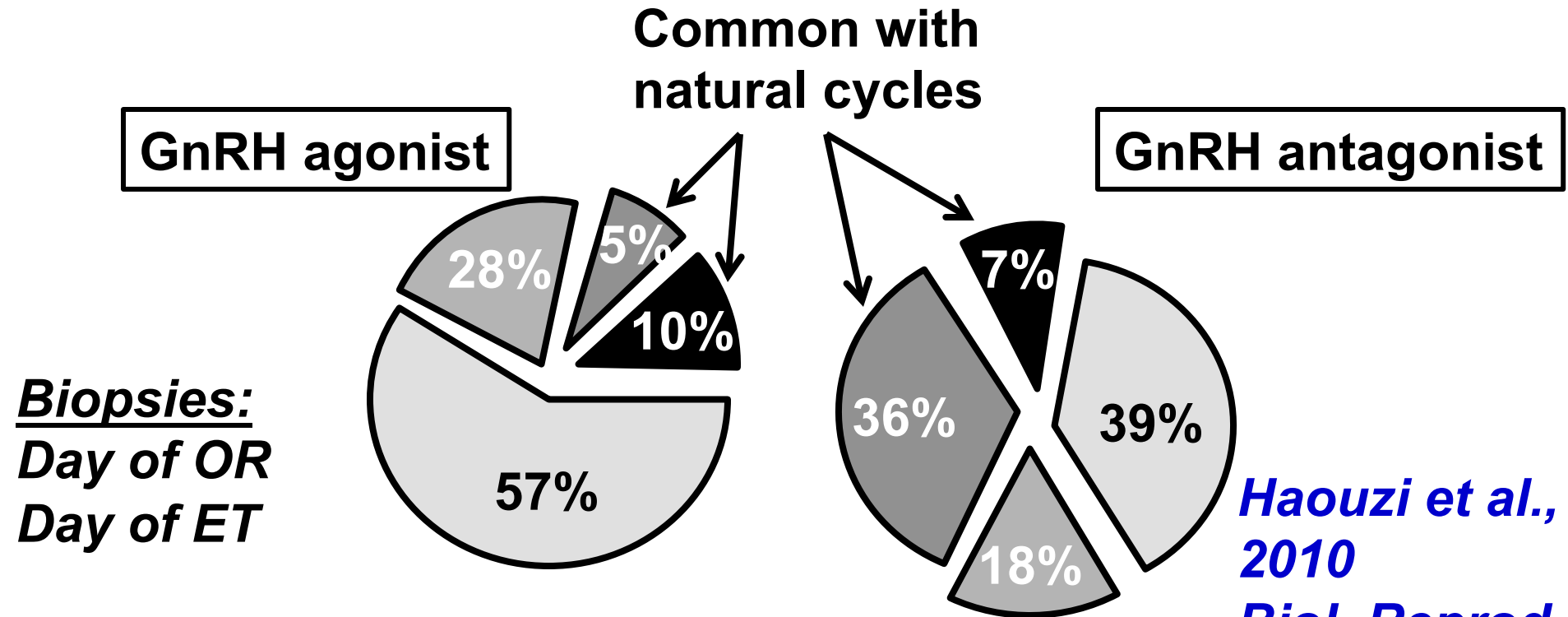
- Endocrine changes
- Endometrial morphology changes
- **Gene transcripts**
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FUNCTIONAL GENOMICS IN THE ENDOMETRIUM (COS cycles)

- 2-day delay in the:
 - *Activation* of 218 implantation genes
 - *Repression* of 133 implantation genes

from the pre-receptive (days LH/HCG+1 until LH/HCG+5) to the receptive phase (day LH+7/HCG+7)

GENES IN COMMON DURING THE RECEPTIVE ENDOMETRIUM



*Haouzi et al.,
2010
Biol. Reprod.
82, 679-686*

- Up-regulated genes (GnRH analogues)
- Down-regulated genes (GnRH analogues)
- Up-regulated genes in common with the natural cycle
- Down-regulated genes in common with the natural cycle

FUNCTIONAL GENOMICS IN COS (Endometrial receptivity)

- 140 genes dys-regulated when **serum P4** >1.5 on HCG day (n=6) vs P4<1.5 ng/ml (n=6):
 - 64 up-regulated
 - 76 down-regulated } Day rHCG+7 (biopsy)
- Genes related to cell adhesion, developmental processes, the immune system

OVARIAN STIMULATION (Luteal phase)

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CLINICAL OUTCOME (Impaired endometrium)

**Cryopreservation
(artificial endometrial
preparation)**

	Fresh	
Retrievals	67	70
Blastocyst transfers	53	50
Clin. Pregn. Rate (%)	54.7	84*
Ong. Pregn. Rate (%)	50.9	78*
Early preg, lossess (%)	19.4	13.3
Implantation rate (%)	38.9	70.8**

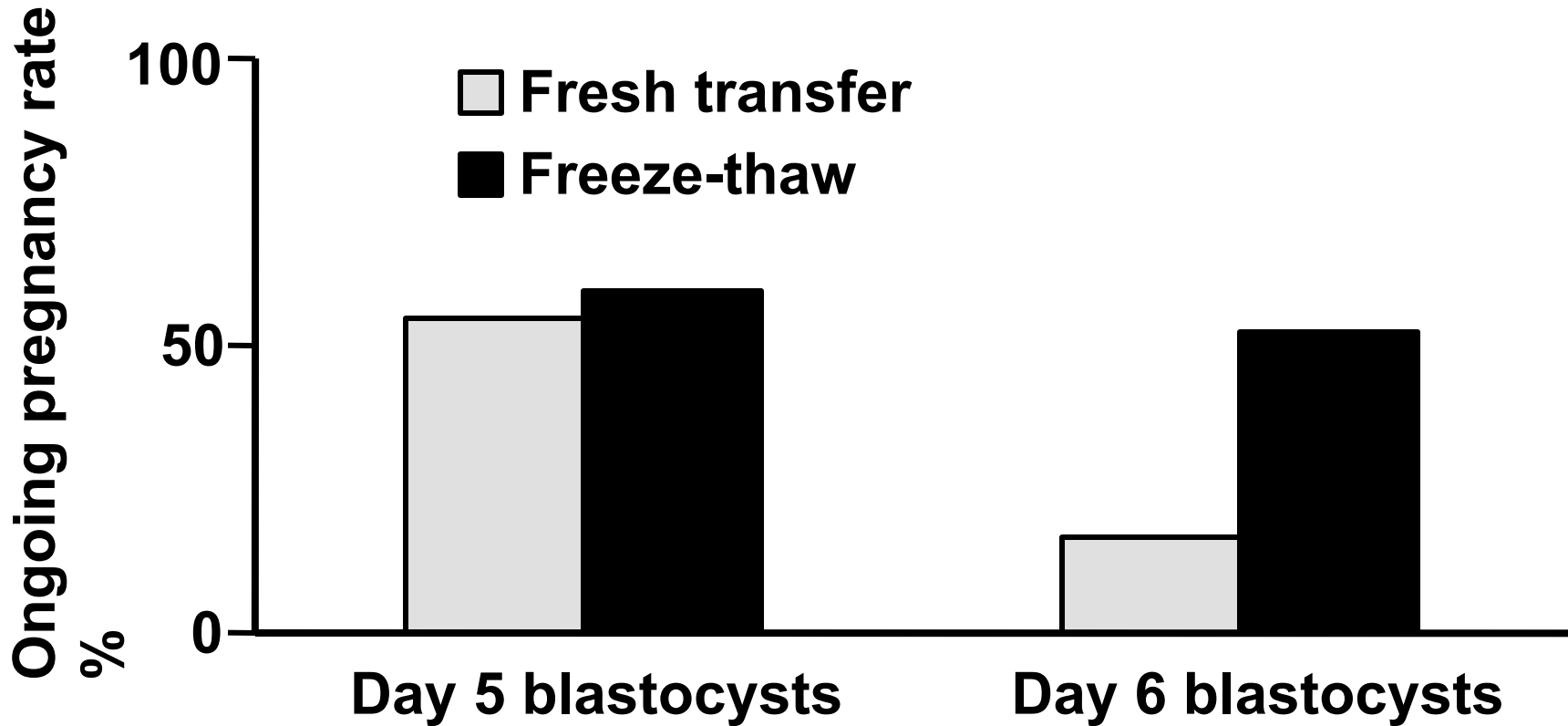
Prospective RCT

*** $P < 0.05$**

**** $P < 0.001$**

***Shapiro et al., 2011
Fertil. Steril. 96, 344-48***

REDUCED PREGNANCY RATE ON DAY 6 (fresh vs thaw)

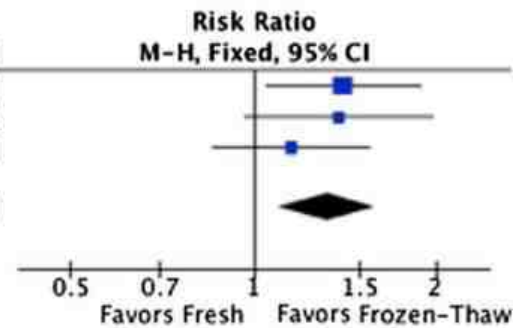


Shapiro et al., 2013
Fertil. Steril. 99, 389-92

FRESH vs FROZEN ET

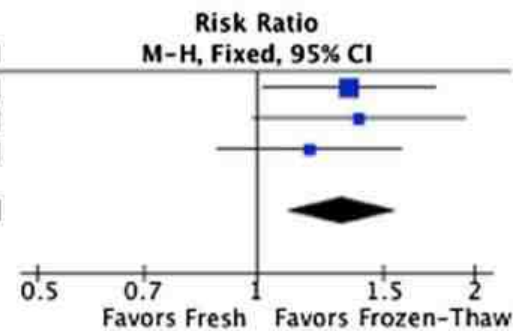
Ong.
Preg.

Study or Subgroup	Frozen-Thawed		Fresh		Weight	Risk Ratio	
	Events	Total	Events	Total		M-H, Fixed, 95% CI	
Aflatoonian 2010	73	187	52	187	46.0%	1.40	[1.05, 1.88]
Shapiro 2011 - Normal	39	70	27	67	24.4%	1.38	[0.97, 1.98]
Shapiro 2011 -High	38	60	34	62	29.6%	1.15	[0.86, 1.55]
Total (95% CI)		317		316	100.0%	1.32	[1.10, 1.59]
Total events	150		113				
Heterogeneity: $\text{Chi}^2 = 1.03$, $\text{df} = 2$ ($P = 0.60$); $I^2 = 0\%$							
Test for overall effect: $Z = 3.00$ ($P = 0.003$)							



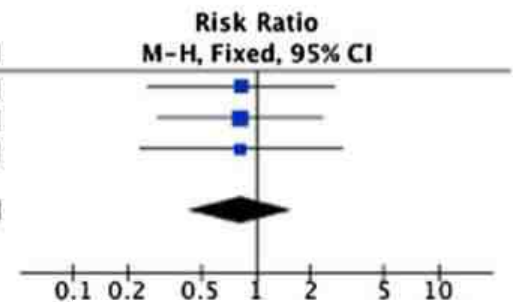
Clin.
Preg.

Study or Subgroup	Frozen-Thawed		Fresh		Weight	Risk Ratio	
	Events	Total	Events	Total		M-H, Fixed, 95% CI	
Aflatoonian 2010	78	187	58	187	47.9%	1.34	[1.02, 1.77]
Shapiro 2011 - Normal	42	70	29	67	24.5%	1.39	[0.99, 1.94]
Shapiro 2011 -High	39	60	34	62	27.6%	1.19	[0.88, 1.59]
Total (95% CI)		317		316	100.0%	1.31	[1.10, 1.56]
Total events	159		121				
Heterogeneity: $\text{Chi}^2 = 0.60$, $\text{df} = 2$ ($P = 0.74$); $I^2 = 0\%$							
Test for overall effect: $Z = 3.04$ ($P = 0.002$)							



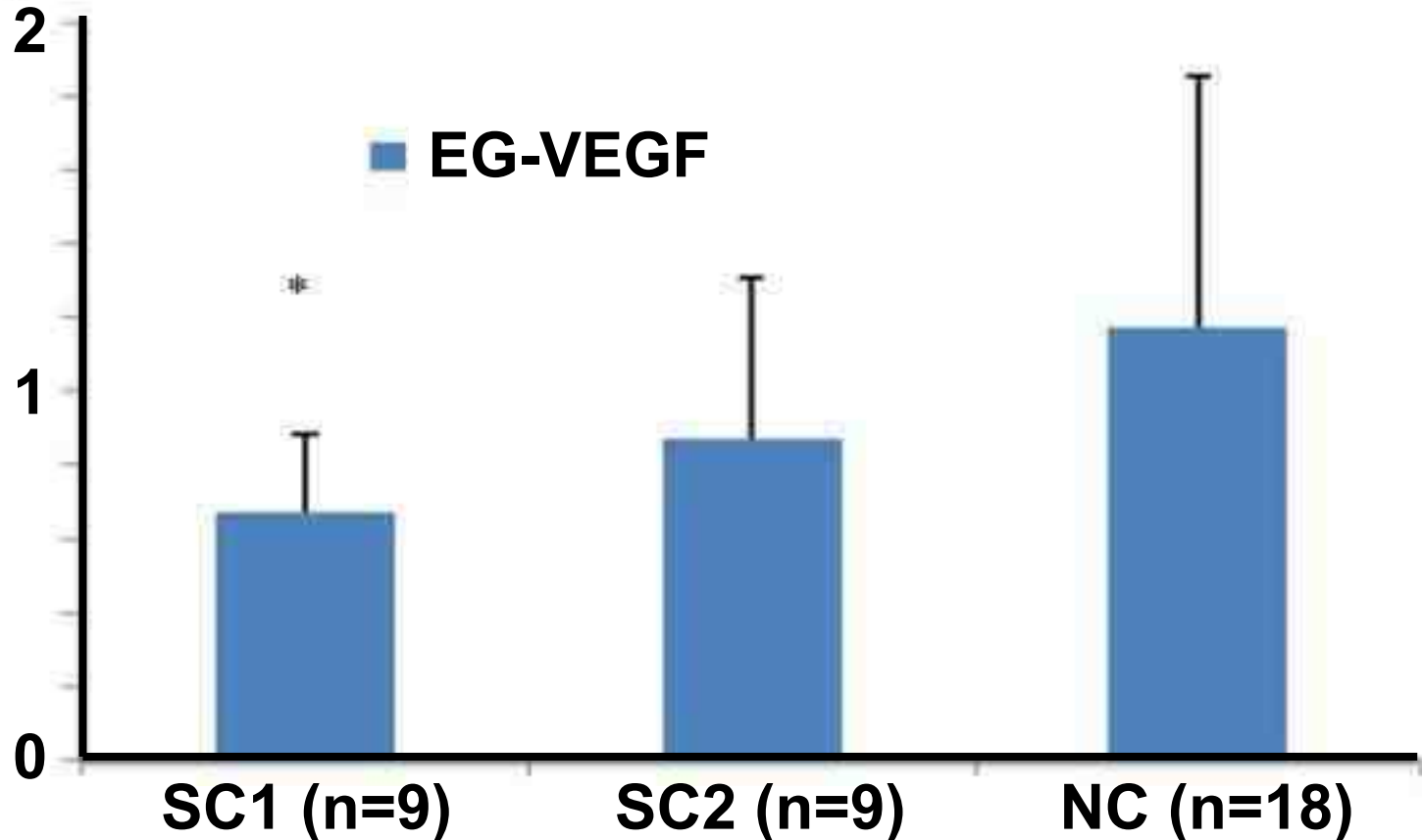
Misc.

Study or Subgroup	Frozen-Thawed		Fresh		Weight	Risk Ratio	
	Events	Total	Events	Total		M-H, Fixed, 95% CI	
Aflatoonian 2010	5	187	6	187	33.2%	0.83	[0.26, 2.68]
Shapiro 2011 - Normal	6	70	7	67	39.6%	0.82	[0.29, 2.32]
Shapiro 2011 -High	4	60	5	62	27.2%	0.83	[0.23, 2.93]
Total (95% CI)		317		316	100.0%	0.83	[0.43, 1.60]
Total events	15		18				
Heterogeneity: $\text{Chi}^2 = 0.00$, $\text{df} = 2$ ($P = 1.00$); $I^2 = 0\%$							
Test for overall effect: $Z = 0.56$ ($P = 0.57$)							



Favors fresh Favours frozen

PERI-IMPLANTATION ENDOMETRIUM



mRNA
RT PCR

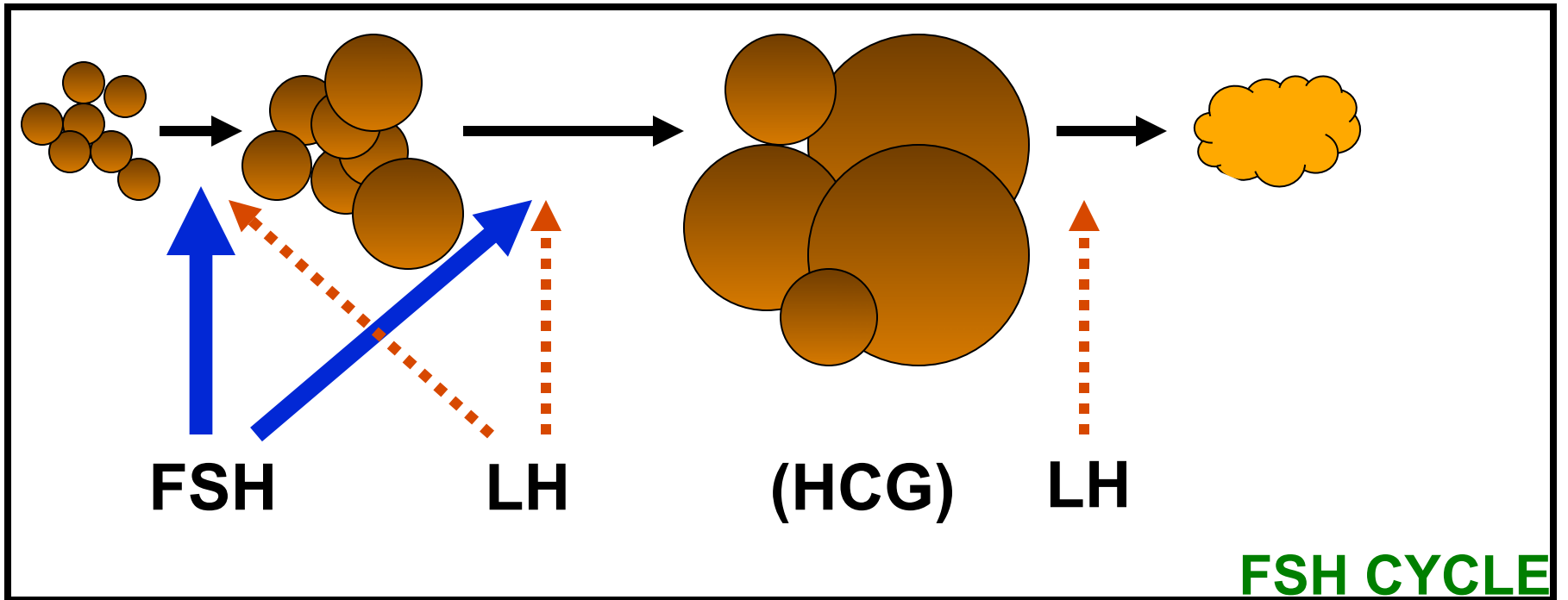
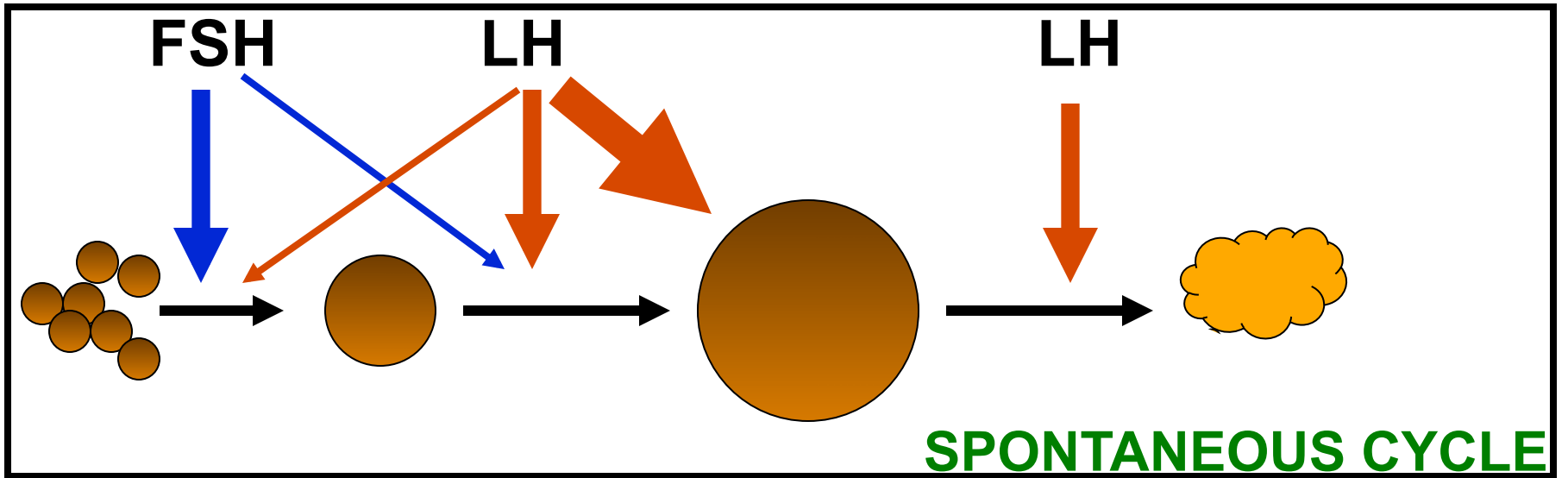
Biopsy 6 days
after ovul. or OR

Xu et al., 2015

Int. J. Clin. Exp. Pathol. 1, 8902-11

OVARIAN STIMULATION (Luteal phase)

- Endocrine changes
- Endometrial morphology changes
- Gene transcripts
- Clinical outcome
- **Luteal support**



LUTEAL PHASE SUPPORT

- P4 or HCG higher Ong.PR and LBR than placebo
- GnRH-a added to P4 improves pregnancy outcomes
- HCG increases the OHSS rate
- Addition of E2 does not improve the clinical outcome
- The route of P4 administration does not affect the outcome

Van der Linden et al., 2015

Cochrane Database Syst. Rev. Jul 7;7:CD009154

TAKE HOME MESSAGES

- **In stimulated cycles:**
 - **LH levels are markedly suppressed in the luteal phase**
 - **Morphological changes, mainly of advanced endometrial maturation (histology, pinopodes), take place**
 - **Changes in functional genomics occur**
 - **Transfer of cryopreserved embryos overcomes the severely compromised endometrial receptivity**