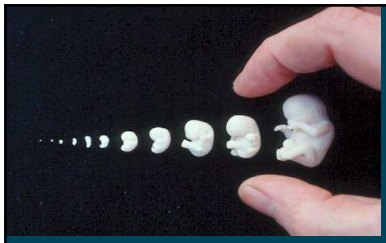


UNIVERSITY OF Southampton
School of Medicine

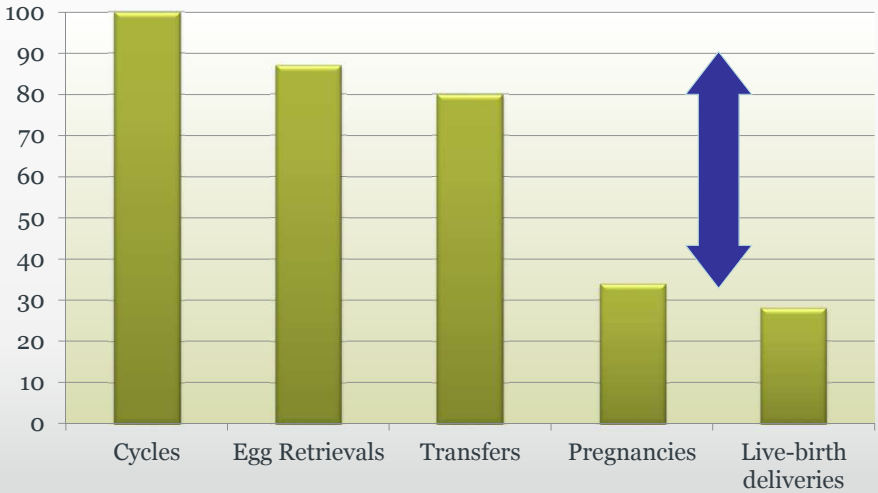
Ovarian stimulation and endometrial receptivity: the body of evidence

Nick Macklon
Professor of Obstetrics and Gynaecology, University of Copenhagen (Roskilde) and University of Southampton, UK.

complete FERTILITY CENTRE SOUTHAMPTON
UNIVERSITY OF Southampton
REGION SJÆLLAND – vi er til for dig
UNIVERSITY OF COPENHAGEN

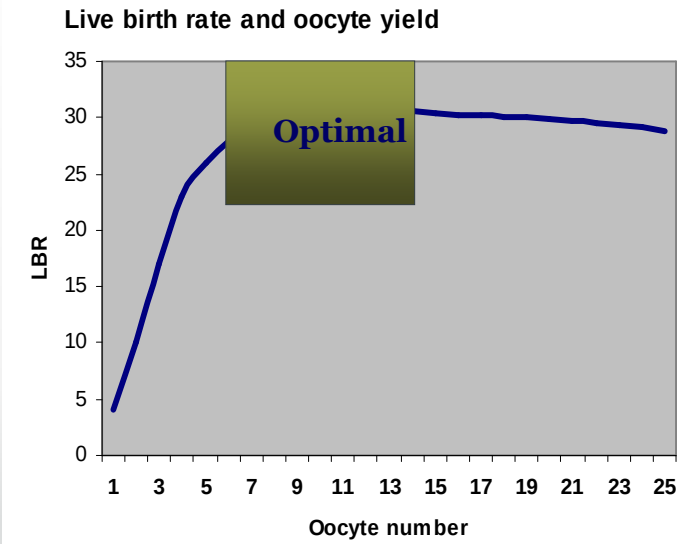
Implantation is rate limiting step in IVF

UNIVERSITY OF Southampton
School of Medicine



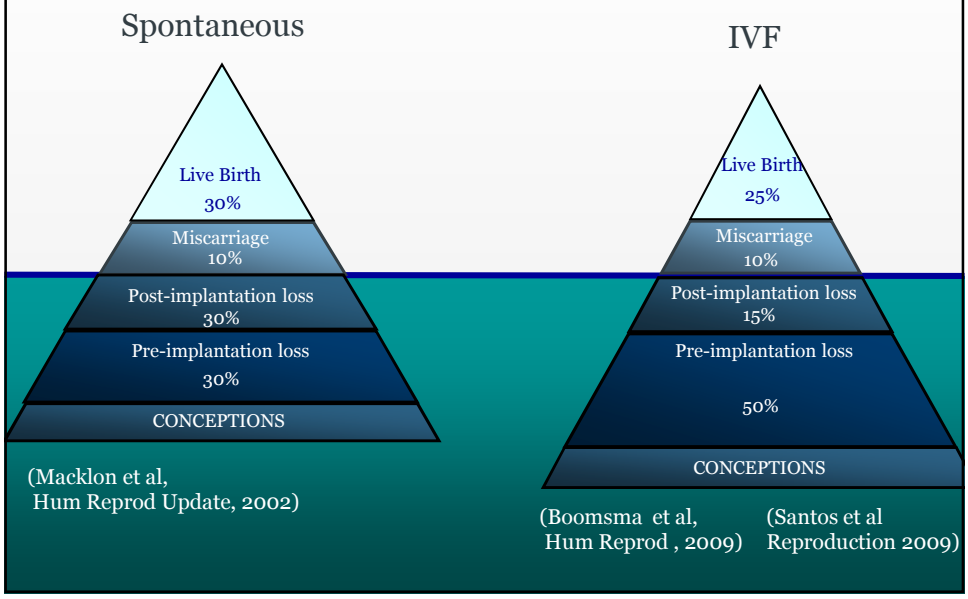
CDC

Optimal number of oocytes after long protocol?



Van der Gaast et al, RBM Online, 2006

The Iceberg of pregnancy loss



(Macklon et al, Hum Reprod Update, 2002)

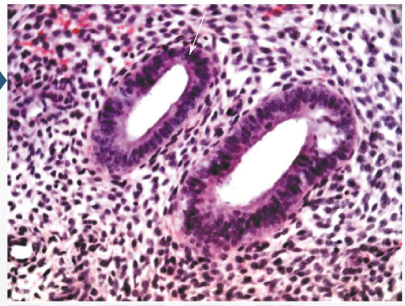
(Boomsma et al, Hum Reprod , 2009)

(Santos et al Reproduction 2009)

Ovarian stimulation damages the endometrium

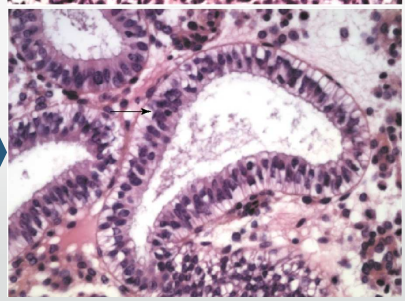
UNIVERSITY OF Southampton School of Medicine

Natural cycle, day of ovulation



When advanced >3 days
NO IMPLANTATION
Kolibianakis, *et al.*
Fertil Steril, 2002.

Stimulated cycle, day of OPU
Secretory features present:
Subnuclear vacuoles
displacing nucleus



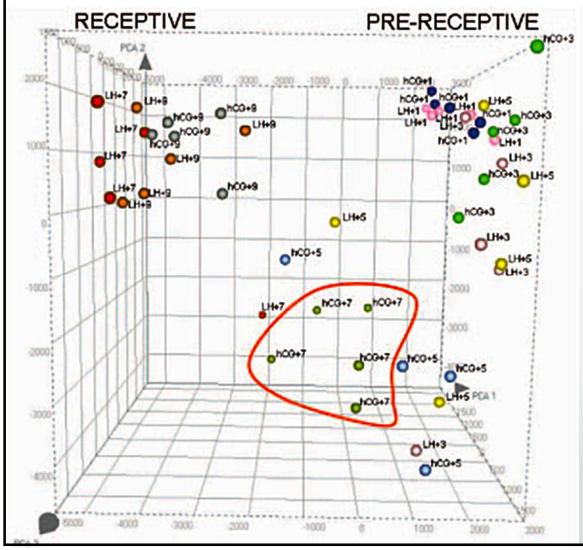
Devroey, *et al.* TEM. 2004;15:84.

ORIGINAL ARTICLE

Endocrine Research

Controlled Ovarian Stimulation Induces a Functional Genomic Delay of the Endometrium with Potential Clinical Implications

José A. Horcajadas, Pablo Minguez, Joaquin Dopazo, Francisco J. Esteban, Francisco Dominguez, Linda C. Giudice, Antonio Pellicer, and Carlos Simón



UNIVERSITY OF Southampton School of Medicine

25 natural cycles

25 stimulated cycles

Biopsies on day
1
3
5
7
after LH rise/hCG

J Clin Endocrinol Metab. 93:4500-4510, 2008.

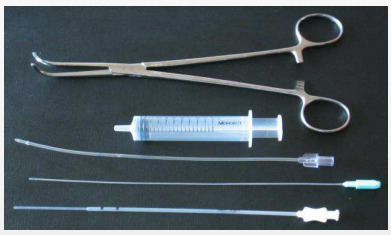
VIEWS AND REVIEWS

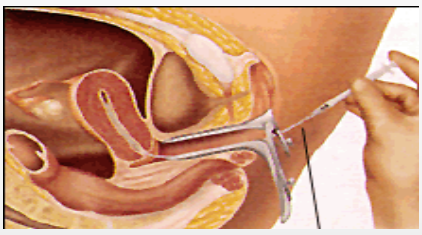
Fertility and Sterility 2013

Uterine secretomics: a window on the maternal-embryo interface

Ying Cheong, M.D.,[a] Carolien Boomsma, Ph.D.,[b] Cobi Heijnen, Ph.D.,[b,c] and Nick Macklon, Ph.D.[a]

[a] Princess Anne Hospital, University of Southampton, Southampton, United Kingdom; [b] University Medical Centre Utrecht, Utrecht, the Netherlands; and [c] M.D. Anderson Cancer Center, University of Texas, Houston, Texas

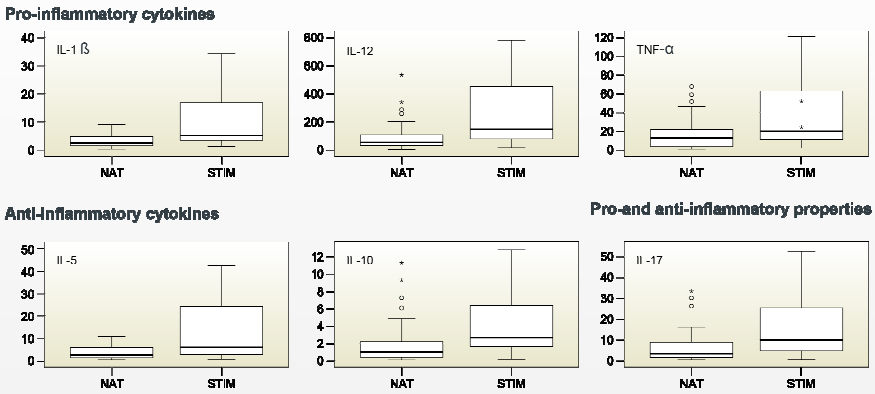




Non-invasive
Simple
Does not disrupt implantation

Ovarian stimulation on intra-uterine cytokine profile

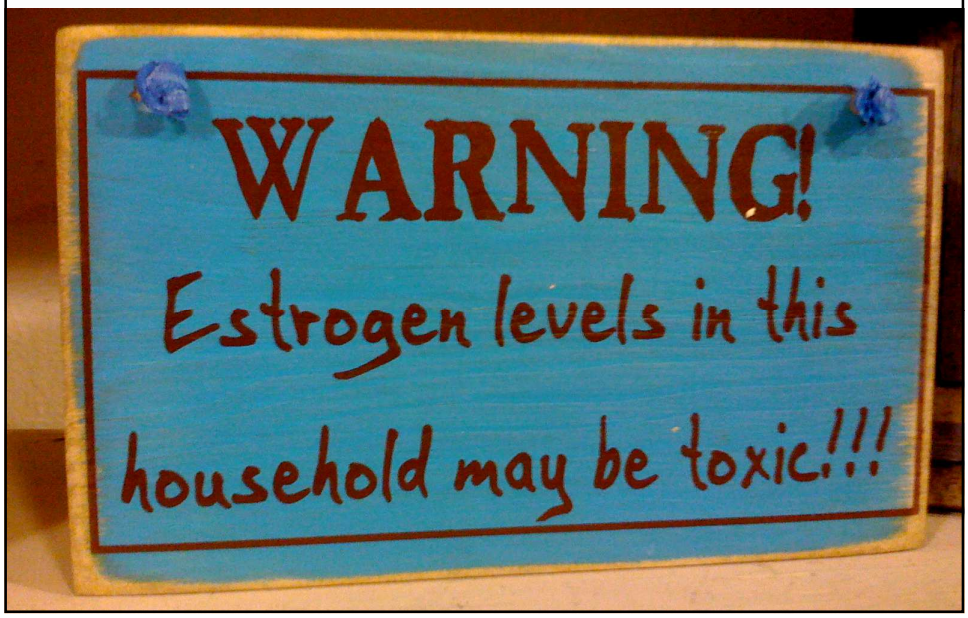
UNIVERSITY OF Southampton
School of Medicine



Multivariable analysis in 203 patients showed significant relations between the number of oocytes retrieved and secretion concentrations of IL-12, Dkk-1 (positive) and VEGF, IL-15 (negative).

Boomsma, *et al. Fertil Steril.* 2010.

UNIVERSITY OF Southampton
School of Medicine



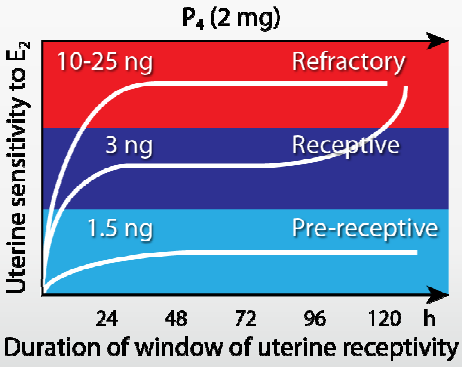
UNIVERSITY OF Southampton
School of Medicine

IVF and the Endometrium

Estrogen is a critical determinant that specifies the duration of the window of uterine receptivity for implantation

Wen-ge Ma[a], Haengseok Song[b], Sanjoy K. Das, Bibhash C. Paria, and Sudhansu K. Dey[c]

A scheme depicting modulation of the window of receptivity in the P4-primed uterus in response to changing estrogen levels. This scheme shows that estrogen at low threshold level extends the window of uterine receptivity for implantation, but higher levels rapidly close this window, transforming the uterus into a refractory state.



Impact of high E2 on P action

UNIVERSITY OF Southampton School of Medicine

- Premature reduction in PR receptors
  (Hadi, *et al. Hum Reprod.* 2004.)
- Reduced PR and ER expression
  (Devroey, *et al. TEM*. 2004.)
- Supraphysiological E2 may increase sensitivity to P action, and thus secretory advancement.
  (Jacobs, *et al. JCEM*. 1987.)
- Accentuated proliferative and early secretory changes before hCG, in absence of rise in Progesterone.
  (Marchini, *et al. Fertil Steril.* 1994)

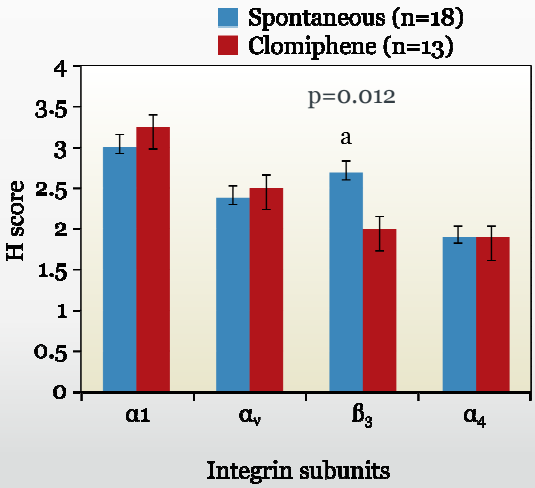
Macklon, *et al. Endocrine Reviews*. 2006.

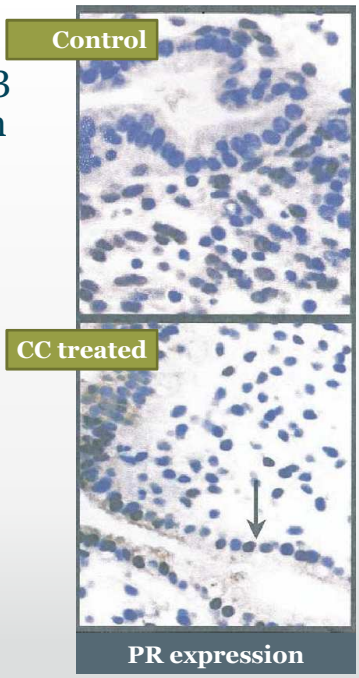
UNIVERSITY OF Southampton School of Medicine

What about clomiphene?

12

Clomiphene reduces Integrin β3 and disrupts PR downregulation





Palomino, *et al. Fertil Steril.* 2005;83:587.

UNIVERSITY OF Southampton
School of Medicine

What about impact of high Progesterone levels?

Relationship between serum P levels on the day of hCG and ongoing pregnancy rate

UNIVERSITY OF Southampton School of Medicine

- A retrospective, observational, single-centre cohort study

Progesterone levels	Pregnancy rate
< or = 1.5 ng/ml	31%
>1.5 ng/ml	19%
	P <0.001

- Multivariate regression analysis showed that **daily FSH** dose, **number of oocytes** and **estradiol values** on the day of hCG administration were positively associated with progesterone levels (P < 0.0001 for all).

Bosch E. *Hum Reprod.* 2010;25:2092–2100.

Hum. Reprod. Advance Access published March 14, 2012
Human Reproduction, Vol.0, No.0 pp. 1–7, 2012
doi:10.1093/humrep/des066

human reproduction ORIGINAL ARTICLE *Reproductive endocrinology*

GnRH-agonist versus GnRH-antagonist IVF cycles: is the reproductive outcome affected by the incidence of progesterone elevation on the day of HCG triggering? A randomized prospective study†

E.G. Papanikolaou[1,2,3,*], G. Pados[1,3], G. Grimbizis[1,3], E. Bili[1,3], L. Kyriazi[3], N.P. Polyzos[4], P. Humaidan[5], H. Tournaye[4], and B. Tarlatzis[1,3]

UNIVERSITY OF Southampton School of Medicine

190 patients

When progesterone exceeded the threshold of 1.5 ng/ml, lower delivery rates:

Agonist group	9.5 versus 31.8%	P= 0.03
Antagonist	14.3 versus 34.3%	P= 0.07

P rise >1.5 ng/ml in 24% of the antagonist group and 23% agonist group

"9 out of 10 patients failed to achieve a clinical pregnancy whenever progesterone levels exceeded the threshold of 1.5 ng/ml"

Progesterone rise on HCG day in GnRH antagonist/rFSH stimulated cycles affects endometrial gene expression

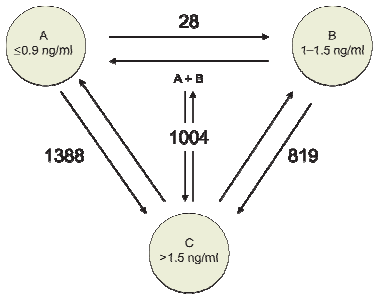
UNIVERSITY OF Southampton School of Medicine

I Van Vaerenbergh [a,*], HM Fatemi [b], C Blockeel [b], L Van Lommel [c], P In't Veld [a], F Schuit [c], EM Kolibianakis [d], P Devroey [b], C Bourgain [a]

Patient's progesterone concentration (ng/ml)	Clinical outcome	Histological dating[a]
Group A (progesterone ≤0.9 ng/ml)		
0.68	Pregnant	+2 days
0.69	Not pregnant	+2 days
0.9	Pregnant	+2 days
Group B (progesterone 1–1.5 ng/ml)		
1.2	Not pregnant	+2 days
1.1	Not pregnant	+2 days
1.44	Pregnant	+3 days
1.1	Pregnant	+3 days
1.05	Not pregnant	+2 days
1.3	Not pregnant	+4 days
Group C (progesterone>1.5 ng/ml)		
1.61	Not pregnant	+4 days
1.8	Not pregnant	+4 days
1.54	Not pregnant	+2 days
1.93	Not pregnant	Late proliferative
1.7	Biochemical pregnancy	+3 days

[a](Advanced) endometrial maturation as compared with the chronological cycle day.

Advancement of gene expression



Differential gene expression between groups of progesterone concentration
(A = ≤0.9 ng/ml; B = 1–1.5 ng/ml; C = >1.5 ng/ml)

Reproductive BioMedicine Online (2011) 22, 263-271.

Human Reproduction, Vol.26, No.7 pp. 1813–1825, 2011
Advanced Access publication on May 2, 2011 doi:10.1093/humrep/der126

human reproduction ORIGINAL ARTICLE *Infertility*

Endometrial receptivity is affected in women with high circulating progesterone levels at the end of the follicular phase: a functional genomics analysis

E. Labarta[1,*], J.A. Martínez-Conejero[2], P. Alamá[1], J.A. Horcajadas[2], A. Pellicer[1], C. Simón[1,2], and E. Bosch[1]

[1]Department of Human Reproduction, Instituto Valenciano de Infertilidad, University of Valencia, Plaza de la Policía Local, 3, 46015 Valencia, Spain [2]Genomix, Valencia, Spain

UNIVERSITY OF Southampton School of Medicine

12 oocyte donors

Progesterone level (On day of hCG)	**# donors**	**# genes significantly dysregulated**	**# gene targets* over-regulated**
>1.5 ng/ml (study group)	6	140	13
<1.5 ng/ml (control group)	6		

*Of the 25 gene targets previously proposed as markers for endometrial receptivity

- Endometrial samples collected 7 days after the hCG injection
- Endometria compared with the control endometria, regardless of the GnRH analogue employed

UNIVERSITY OF Southampton School of Medicine

Beyond Implantation

UNIVERSITY OF Southampton School of Medicine

The endometrium and the baby

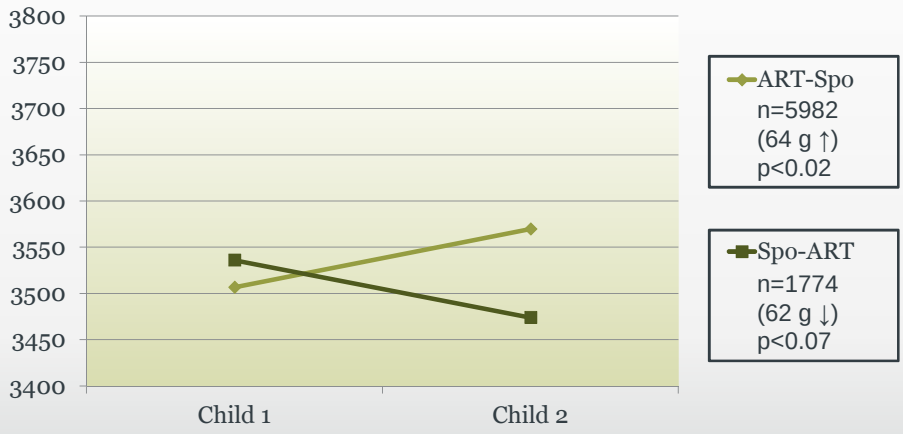
- Perinatal outcome of singleton siblings born after Assisted Reproductive Technology and spontaneous conception

  Danish National Sibling-Cohort study

AIM: Separate the effects of the maternal characteristics and the effects of infertility

Henningsen AA, Pinborg A, Lidegaard Ø, Vestergaard C, Forman JL, Andersen AN

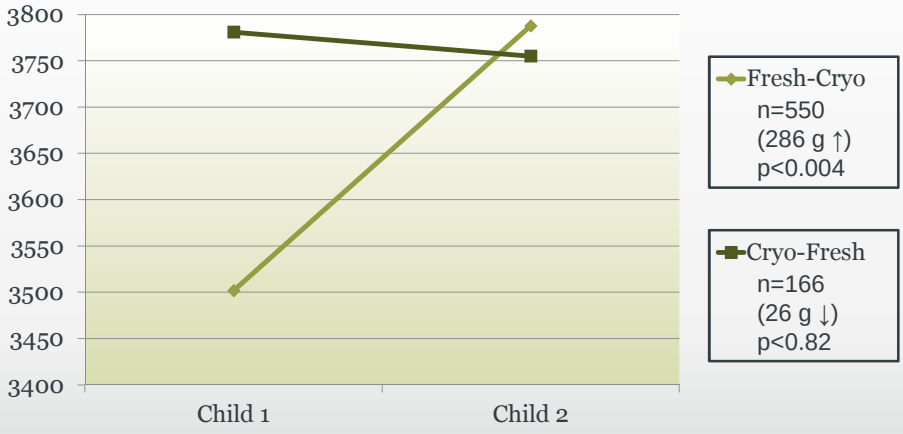
Birthweight (g), adjusted*



*maternal age, parity, year of birth, sex

IVF procedure or Ovarian Stimulation?

Cryo: Birthweight (g), adj.*



*maternal age, parity, year of birth, sex

Freeze all frees all!

UNIVERSITY OF Southampton School of Medicine

- Doctor free to stimulate ovaries without disrupting endometrium
- Women free of OHSS risk
- Embryos free to implant in more physiological environment
- Babies free of impact of ovarian stimulation on development

'E-FREEZE'

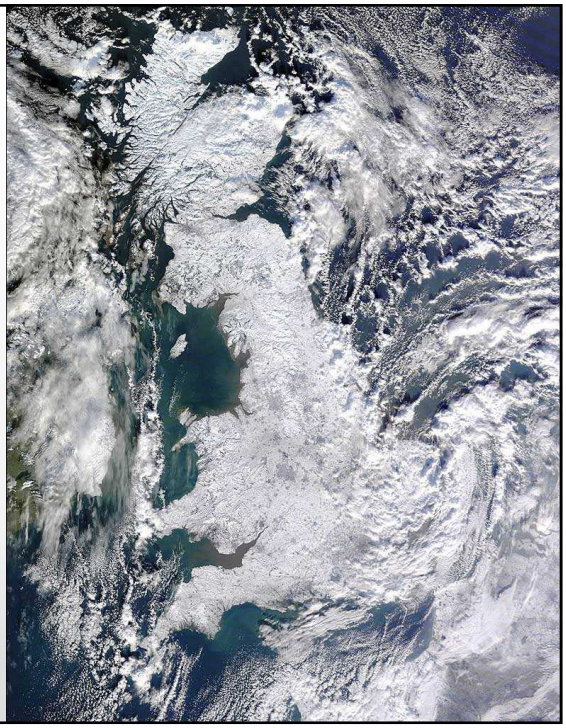
UK Multicentre RCT

•Elective freeze all versus fresh transfer n=918

•Powered for livebirth rate

•Costs, health outcomes etc

•Starts December 2015

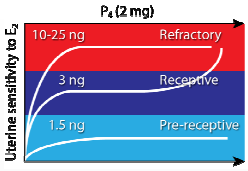


UNIVERSITY OF Southampton
School of Medicine

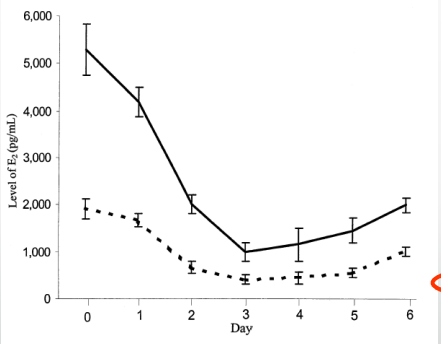
What can we do to ameliorate the impact of ovarian stimulation on the endometrium?

25

Does reducing E2 exposure improve implantation rates?



1. Significant decreases in implantation and pregnancy rates when E2 levels >2500 pg/ml on day of hCG[1]
2. Stepdown protocol improves outcomes in high responders[2]



Reproductive outcome of the step-down cycle versus the previous failed cycle of IVF.

Variable	Previous cycle (n = 24)	Step-down cycle (n = 24)	P value
Patient age (y)	31.1 ± 1.4	31.6 ± 1.2	NS
GnRH analogue (d)	20.2 ± 0.6	22.9 ± 0.7	NS
Days of treatment	9.7 ± 0.4	10.6 ± 0.1	NS
Ampules of gonadotropins	27.2 ± 2.1	22.4 ± 0.8	NS
E_2 level (pg/mL)	5,770 ± 650	1,919 ± 477	.001
No. of oocytes	24.0 ± 1.9	18.1 ± 2.1	.001
Fertilization rate (%)	71.2	74.2	NS
No. of embryos transferred	3.8 ± 0.2	3.3 ± 0.2	NS
No. of embryos frozen	6.2 ± 1.0	2.3 ± 0.7	.002
Pregnancy rate (%)	0	64.2	.0001
Implantation rate (%)	0	29.3	.0001

Note: Values are expressed as means ± SE unless otherwise indicated. NS = not significant.

1. Simon, *et al. Hum Reprod.*1995;10:2432.
2. Simon, *et al. Fertil Steril.*1998;70:234.

Does milder stimulation reduce estradiol and progesterone levels at the end of the follicular phase?

University of Southampton School of Medicine

Follicular Phase Endocrine Characteristics during Ovarian Stimulation and GnRH Antagonist Cotreatment for IVF: RCT Comparing recFSH Initiated on Cycle Day 2 or 5

Christophe Blockeel,* Monique D. Sterrenburg,* Frank J. Broekmans, Marinus J. C. Eijkemans, Johan Smitz, Paul Devroey, and Bart C. J. M. Fauser

Centre for Reproductive Medicine (C.B., J.S., P.D.), Universitair Ziekenhuis Brussel, 1090 Brussels, Belgium; Department of Reproductive Medicine and Gynecology (M.D.S., F.J.B., M.J.C.E., B.C.J.M.F.) and Julius Centre for Health Sciences and Primary Care (M.J.C.E.), University Medical Centre Utrecht, 3508 GA Utrecht, The Netherlands

Blockeel C, *et al. JCEM*. 2011;96:1122-1128.

Follicular Phase Endocrine Characteristics during Ovarian Stimulation and GnRH Antagonist Cotreatment for IVF: RCT Comparing recFSH Initiated on Cycle Day 2 or 5

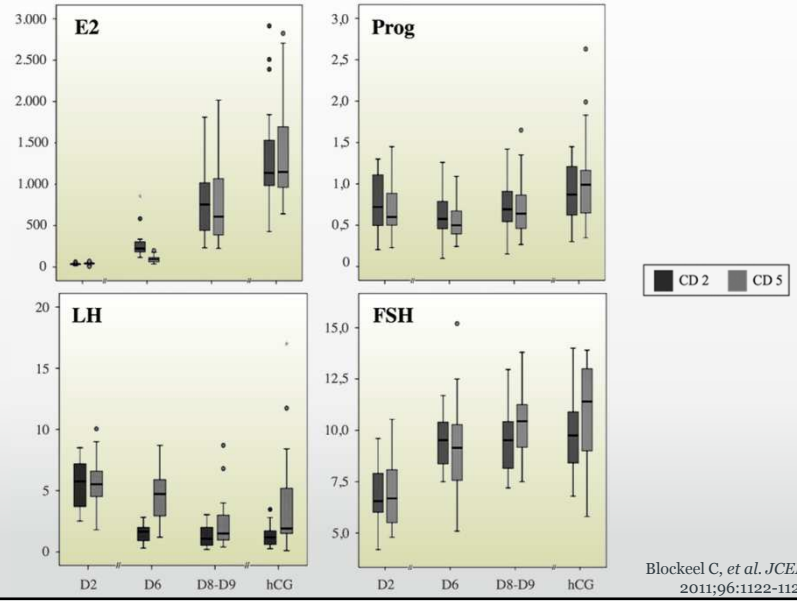
Christophe Blockeel,* Monique D. Sterrenburg,* Frank J. Broekmans, Marinus J. C. Eijkemans, Johan Smitz, Paul Devroey, and Bart C. J. M. Fauser

Follicular characteristics and cycle outcome measures

	CD2 group (n = 33)	CD 5 group (n = 39)	P
Total dose of recFSH (IU)	1364 ± 226	1177 ± 295	<0.01
recFSH duration (days)	9.1 ± 1.5	7.8 ± 2.0	<0.01
Duration follicular phase (days)	10.1 ± 1.5	11.9 ± 2.0	<0.01

Blockeel C, *et al. JCEM*. 2011;96:1122-1128.

JCEM THE JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM
APRIL 2011 • VOLUME 96 • NUMBER 04



Blockeel C, *et al. JCEM.* 2011;96:1122-1128.

UNIVERSITY OF Southampton
School of Medicine

Is there a role for adjuvant medication to reduce supraphysiological E and P action?

UNIVERSITY OF Southampton School of Medicine

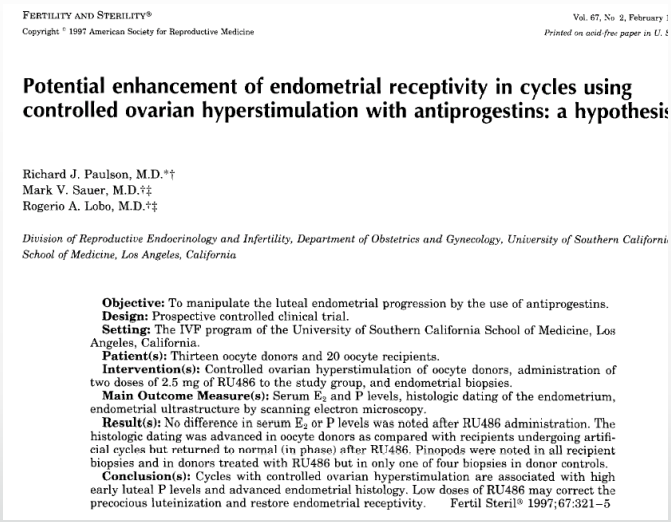
Aromatase inhibitors?

- Reduce E2 levels at end of follicular phase.
- Used in IVF to reduce E 2 exposure for Breast Cancer patients.
- Published data available in poor responders and cancer patients.
- No evidence of increased risk fetal anomalies (Tulandi, *et al.*)
- 'RIOT' study commenced in Copenhagen

UNIVERSITY OF Southampton School of Medicine

Progesterone Antagonists?

UNIVERSITY OF Southampton
School of Medicine



FERTILITY AND STERILITY®
Copyright © 1997 American Society for Reproductive Medicine
Vol. 67, No. 2, February
Printed on acid-free paper in U.

Potential enhancement of endometrial receptivity in cycles using controlled ovarian hyperstimulation with antiprogestins: a hypothesis

Richard J. Paulson, M.D.*†
Mark V. Sauer, M.D.†‡
Rogerio A. Lobo, M.D.†‡

Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Southern California School of Medicine, Los Angeles, California

Objective: To manipulate the luteal endometrial progression by the use of antiprogestins.
Design: Prospective controlled clinical trial.
Setting: The IVF program of the University of Southern California School of Medicine, Los Angeles, California.
Patient(s): Thirteen oocyte donors and 20 oocyte recipients.
Intervention(s): Controlled ovarian hyperstimulation of oocyte donors, administration of two doses of 2.5 mg of RU486 to the study group, and endometrial biopsies.
Main Outcome Measure(s): Serum E_2 and P levels, histologic dating of the endometrium, endometrial ultrastructure by scanning electron microscopy.
Result(s): No difference in serum E_2 or P levels was noted after RU486 administration. The histologic dating was advanced in oocyte donors as compared with recipients undergoing artificial cycles but returned to normal (in phase) after RU486. Pinopods were noted in all recipient biopsies and in donors treated with RU486 but in only one of four biopsies in donor controls.
Conclusion(s): Cycles with controlled ovarian hyperstimulation are associated with high early luteal P levels and advanced endometrial histology. Low doses of RU486 may correct the precocious luteinization and restore endometrial receptivity. Fertil Steril® 1997;67:321–5

UNIVERSITY OF Southampton
School of Medicine

Conclusions

- Despite embryo selection, implantation rates after IVF are lower than after spontaneous conceptions
- Ovarian stimulation disrupts the endometrium and intra-uterine environment
- Clomiphene, supraphysiological estradiol and progesterone all implicated
- Freeze all strategies await assessment
- Endocrine modulation strategies being explored.

Acknowledgements

Alex Kermack
Keith Godfrey
Philip Calder
Franchesca Houghton
Tom Fleming

Gijs Teklenburg
Carolien Boomsma
Lotte Weimar
Frank Holstege
Cobi Heijnen
Bart Fauser

Birgit Gellersen
Jan Brosens

Xiao Shilong
Hywel Morgan
Ying Cheong

Morten Petersen
Soeren Ziebe
Sven Skouby
Thomas Hvidd