UMC Utrecht

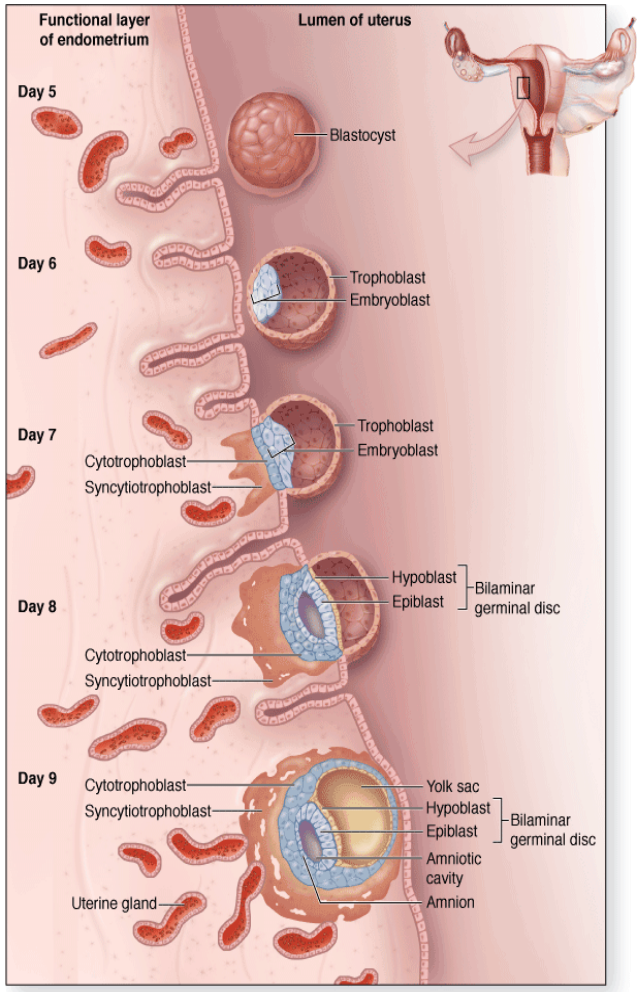
To SCRaTCH or not to scratch
that's the question

Helen Torrance
Associate Professor Reproductive Medicine
University Medical Center Utrecht

KLEM wetenschapsmiddag 12 januari 2018

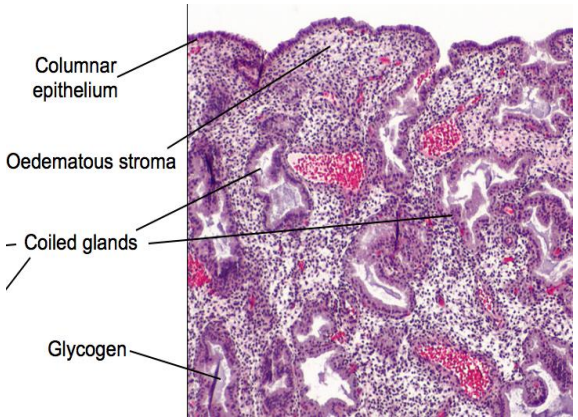
SCRATCH-OFO

Successfull implantation

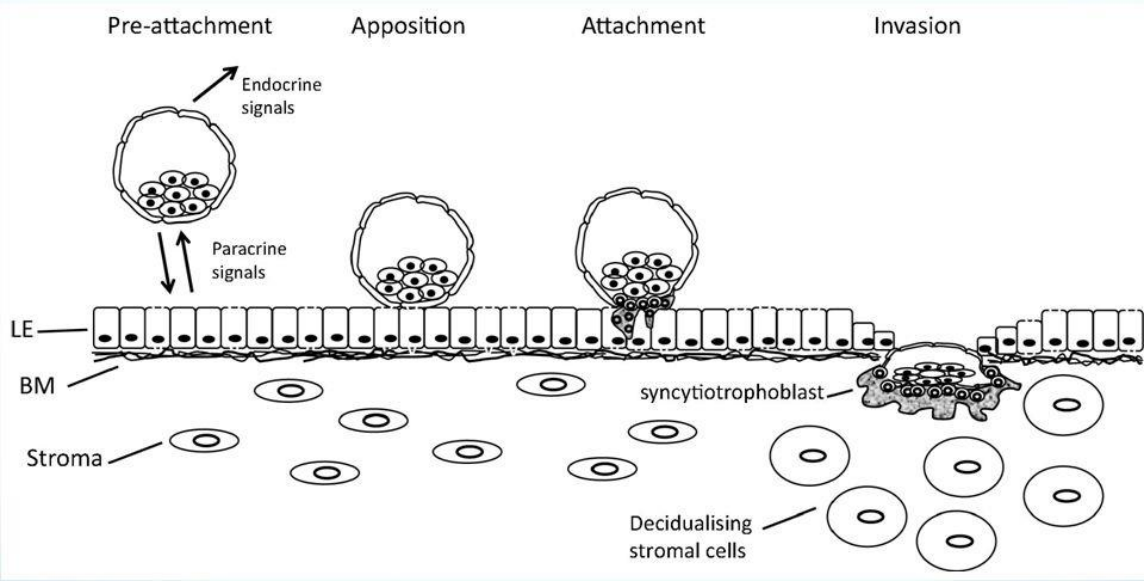


is a multi-step process that requires synchronous development of both the endometrium and embryo.

The intricate dialogue between the embryo and endometrium is possible during a specific timeframe: the window of implantation (WOI).



Multi step process during WOI



Sharkey
RBMO 2013

Zona pellucida rupture

Apposition

Endometrial crypt, site of lysis of the zona pellucida,
Contact between trophoblast and decidua

Adhesion

Protrusions of trophoblast cells penetrating the endometrium

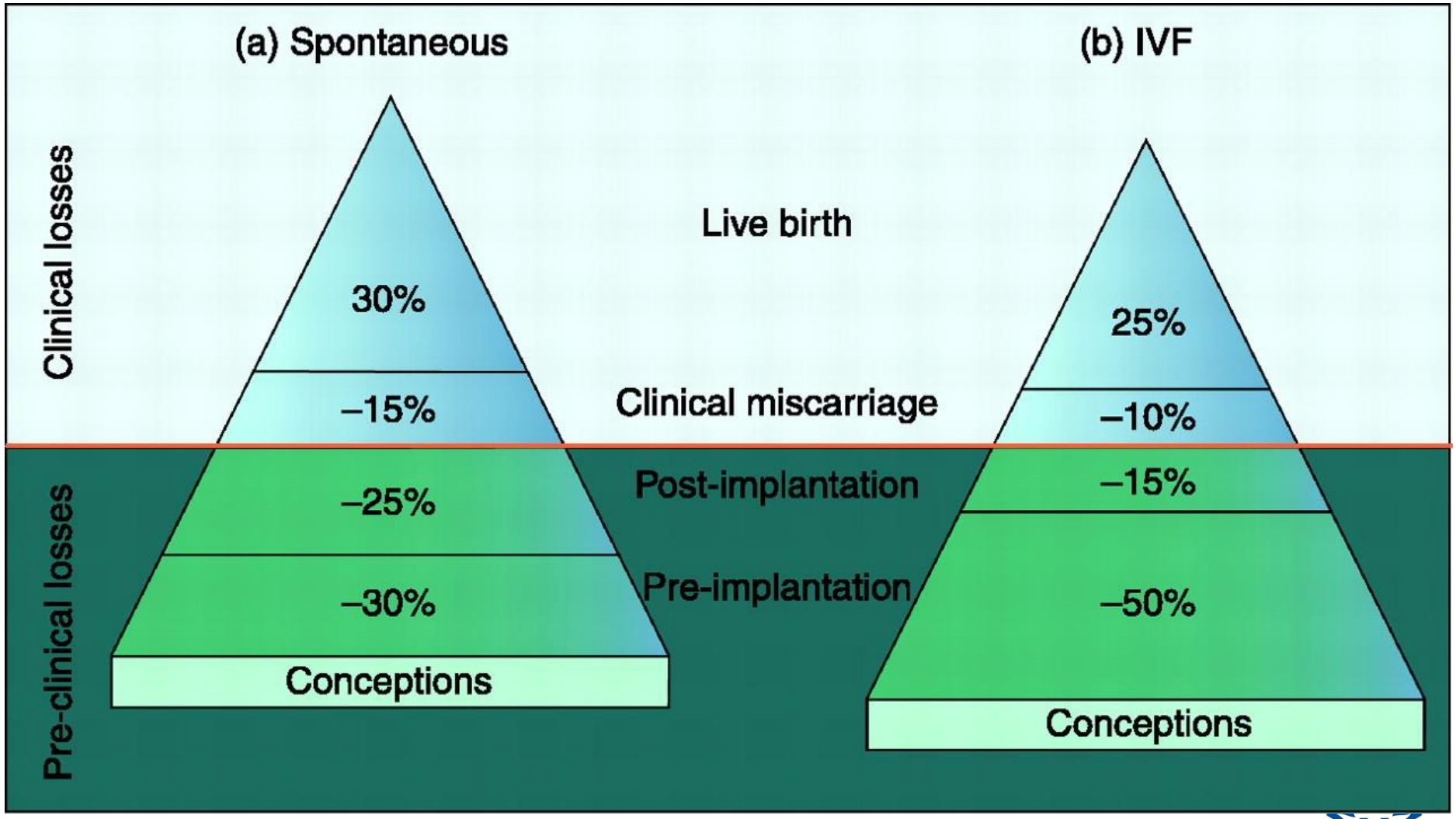
Invasion

Further establishment of blastocyst in endometrium.
Penetrating trophoblast differentiates into syncytiotrophoblast that eventually penetrate the stroma and develop into chorionic villi & placenta.
Cytotrophoblast

Important factors facilitating these stages:

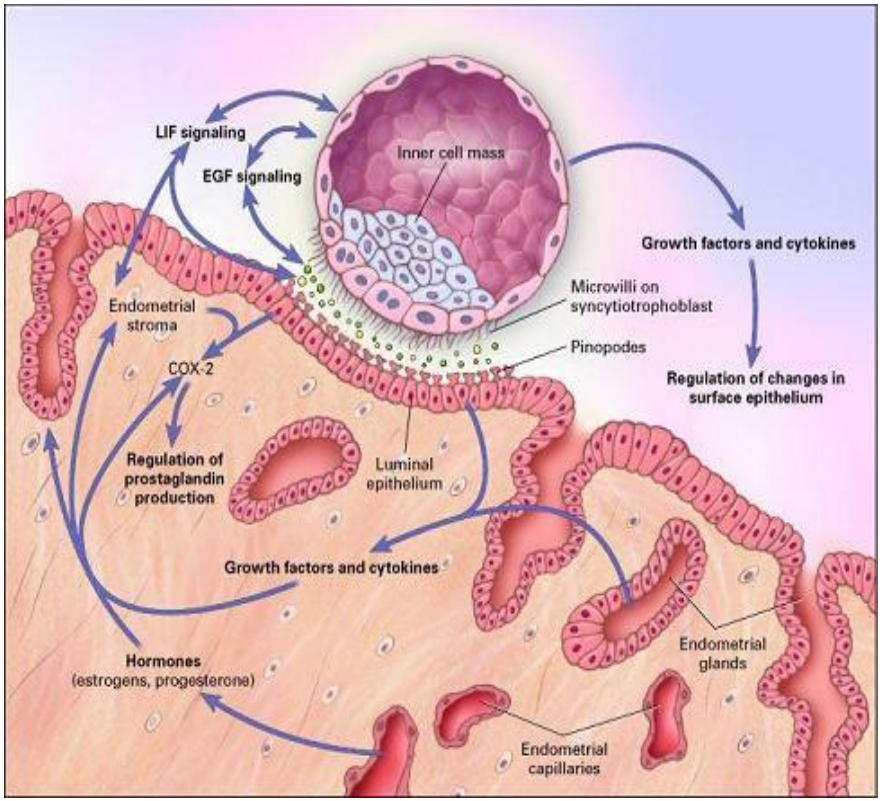
Uterine plasminogen
Cellular adhesion molecules family (CAM) including integrins, cadherins, selectins, immunoglobins
Mucins
Cytokines
LIF
Interleukins
Colony stimulating factor
Prostaglandins
Proteoglycan receptors

‘Iceberg of conception loss’



M. Santos et al Reproduction 2009

Repeated (pre-/post-) implantation failure (RIF) causes considerable emotional, physical and financial burden in women undergoing IVF

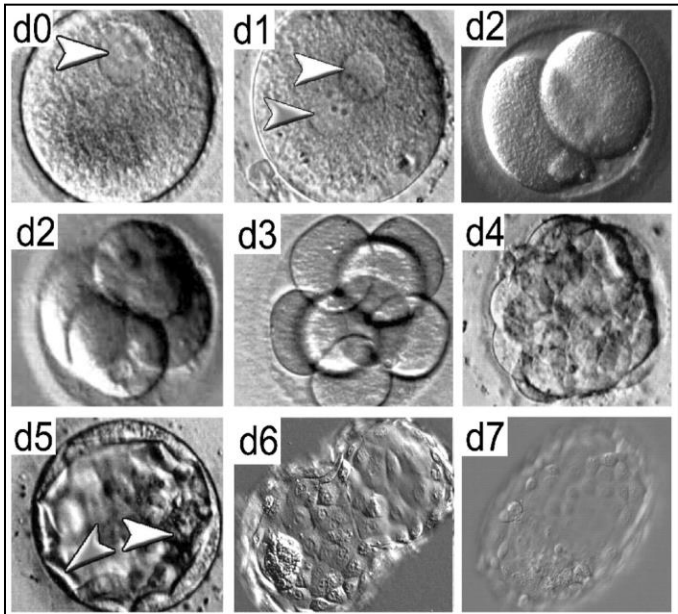


But what is RIF? exactly

RIF has been defined as:

"the absence of implantation after three or more transfers of high quality embryos or after placement of 10 or more embryos [2,3]"

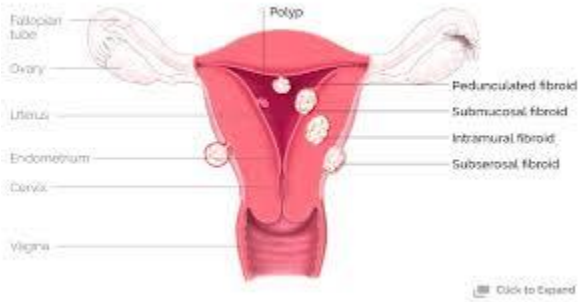
No consensus exists and varying definitions are used in scientific literature.

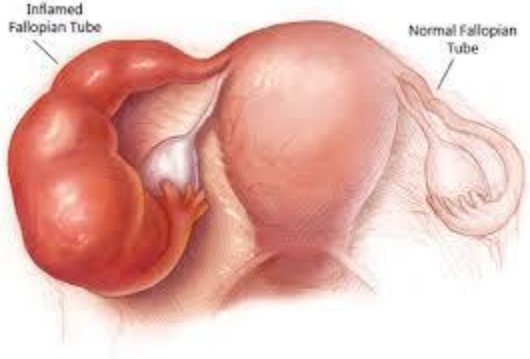


[2] Thornhill *et al. ESHRE. Hum. Reprod.* 2005

[3] Simon et al. *Fertil. Steril.* 2012

What causes ‘RIF’?



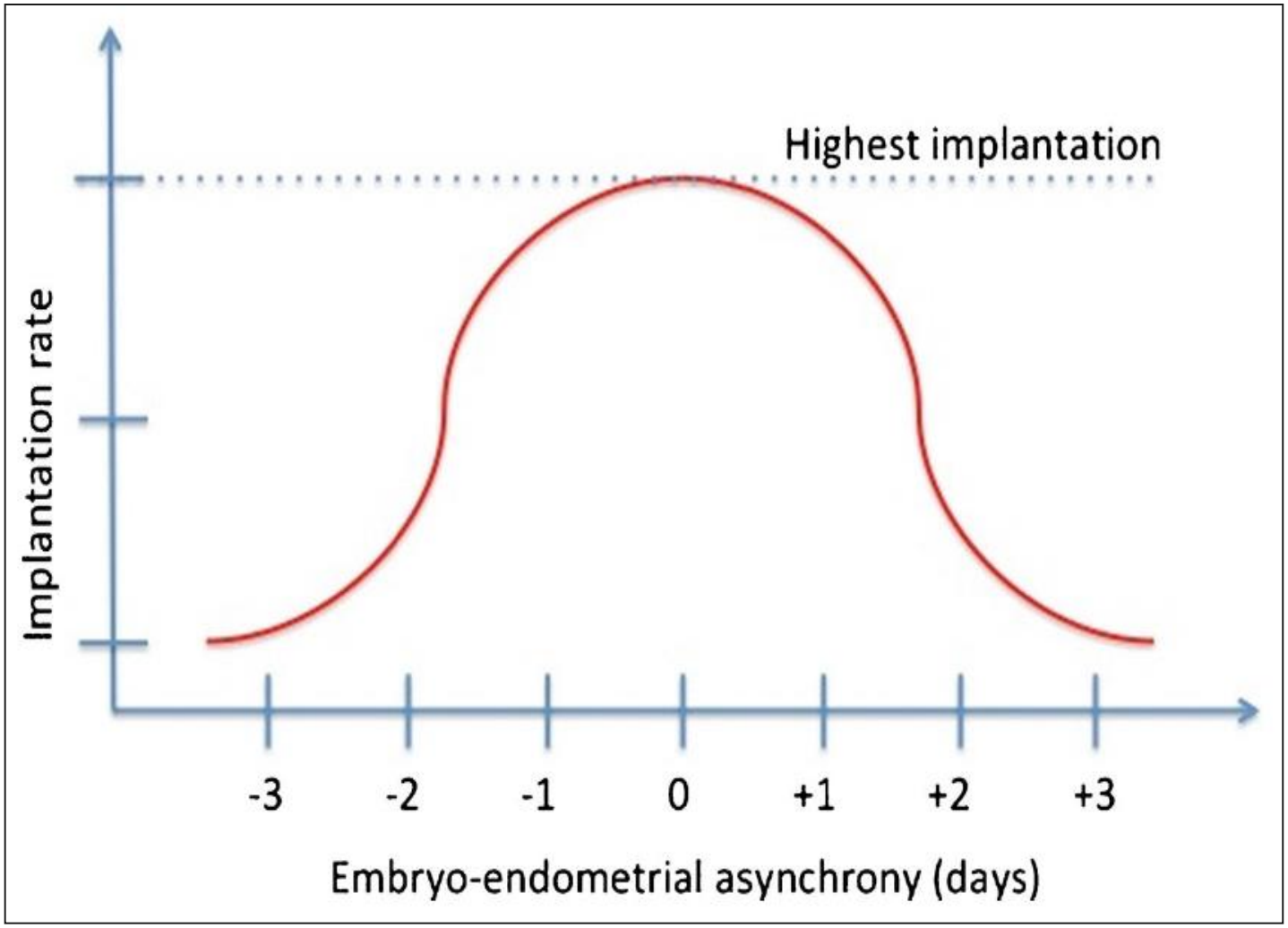


Factors that may contribute to RIF include:

- Poor embryo quality - aneuploidy
- Advanced maternal age
- Uterine cavity abnormalities
- Tubal fluid accumulation
- Poor execution of embryo transfer
- Poor endometrial receptivity: timing/structural?

However, in the majority no clear cause for RIF can be identified (Koot & Macklon *Curr. Opin. Obstet. Gynecol.* 2013)

Does a timing issue exist?



The ERA test

Summary of the diagnostic and clinical outcomes of repeated implantation failure (RIF) and comparison group patients following the endometrial receptivity array (ERA) test.

	RIF	Control
No. of patients	85	25
Age (y)	38.4 ± 4.7	39.9 ± 5.1
No. of R ERA/total analyzed	63/85 (74.1)	22/25 (88.0)
No. of previous failed cycles	4.8 ± 2.1	0.5 ± 0.5
No. of NR ERA/total analyzed (%)	22/85 (25.9)	3/25 (12.0)
No. of previous failed cycles	5.0 ± 1.8	0.3 ± 0.6

Increased percentage of WOI displacement in RIF patients, based on the **E**ndometrium **R**eceptivity **A**rray (ERA) test, comprising 238 expressed genes.

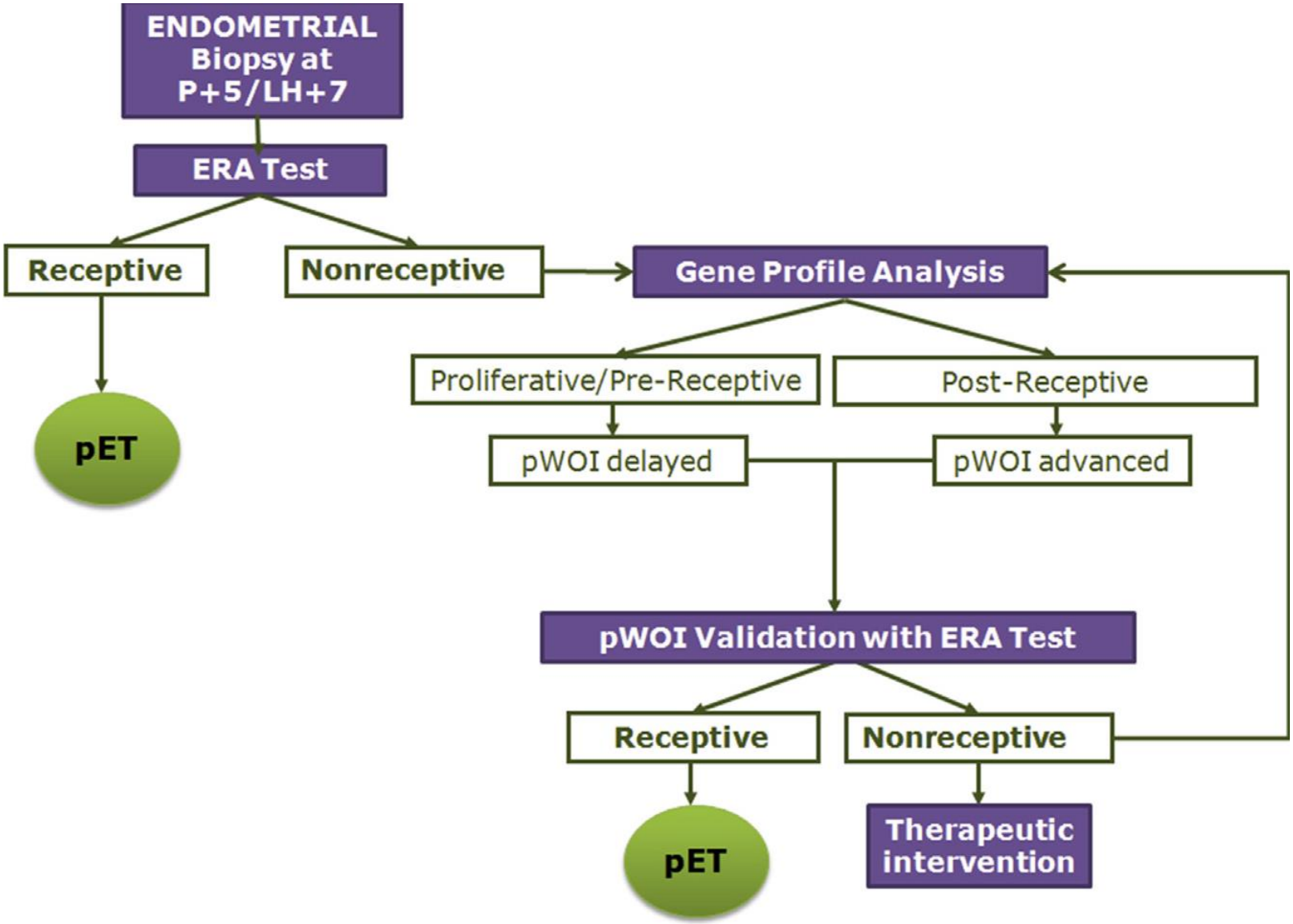
Ruiz- Alonso, FS 2013

If non-receptive:

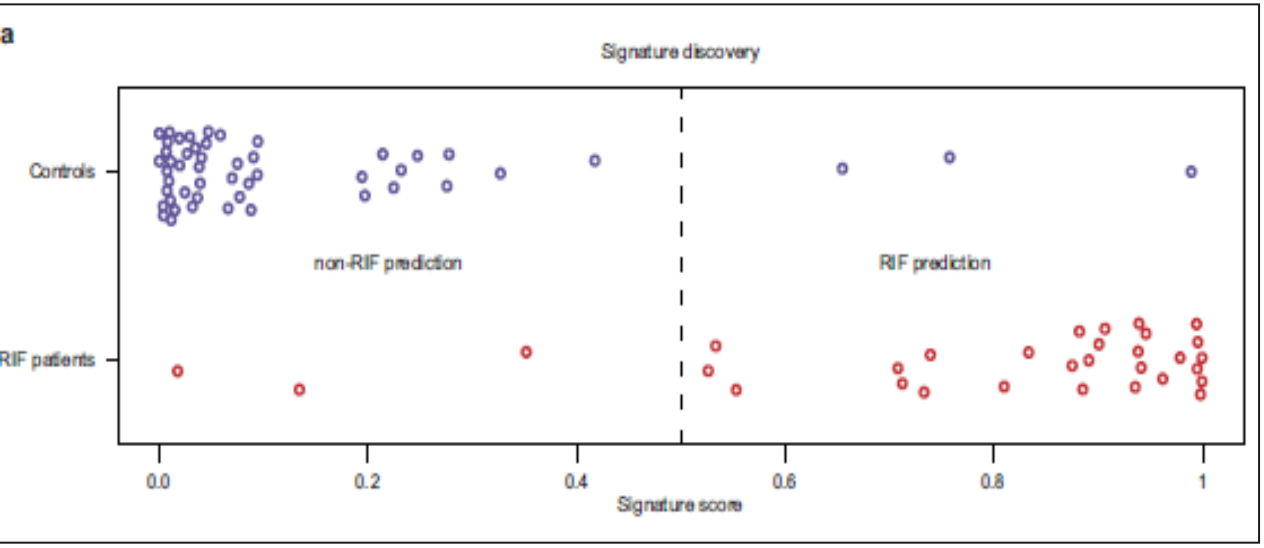
A Clinical outcome of non receptive RIF and control patients that underwent pET

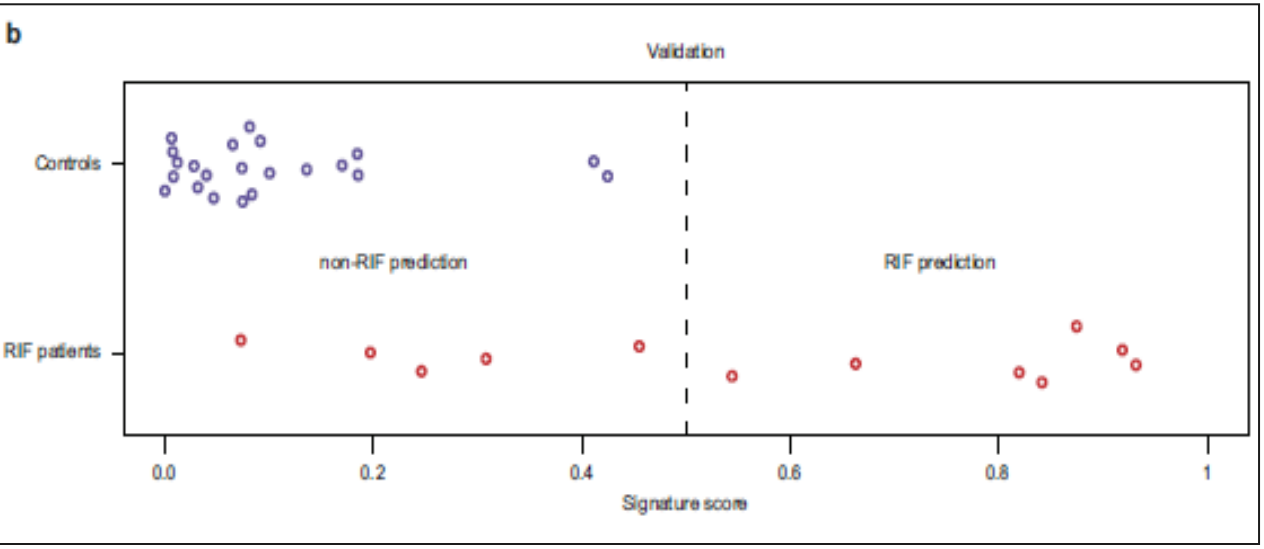
	Non Receptive
No. of patients	25
No. of previous failed cycle RIF Patients	5.0±1.8
No. of previous failed cycle Control Patients	0.3±0.6
ERA Prediction	
Pre-receptive	21/25 (84.0)
Post-receptive	4/25 (16.0)
2nd ERA at the specified day (P+4;P+6;P+7;LH+9) [a]	18
Months between 1st and 2nd ERA	2.6±2.8
2nd ERA Receptive at the specified day	15
Patients with pET[b] after 2nd RECEPTIVE ERA	8
Months between 2nd RECEPTIVE ERA and pET	1.8±0.7
Implantation rate using pET	5/13 (38.5)
Pregnancy rate using pET	4/8 (50.0)
Biochemical pregnancies (%)	0/4 (0.0)
Clinical abortions (%)	0/4 (0.0)

Therapeutic option for RIF?



Endometrial gene expression profile





Mid-luteal phase endometrial biopsies in RIF (n=43) and controls (n=72).

RNA microarray gene expression.

Discovery set (n = 81): 303 signature genes predictive of RIF.

Validation set (n=34)

Koot et al Science 2016

Diagnostic accuracy

Metric	Signature Discovery	Validation
NPV, % (95% CI)	94.0 (83.8–97.9)	81.5 (63.3–91.8)
PPV, % (95% CI)	90.3 (75.1–96.7)	100 (64.6–100)
Sensitivity, % (95% CI)	90.3 (75.1–96.7)	58.3 (32.0–80.7)
Specificity, % (95% CI)	94.0 (83.8–97.9)	100 (85.1–100)
Overall accuracy, % (95% CI)	92.6 (84.8–96.6)	85.3 (69.9–93.6)
P	3.83×10^{-13}	0.0147

External validation needs to be performed

Counselling tool?

So, what is RIF and what can we do about it?

It is a putative "clinical condition"

that occurs in many couples,

that we do not fully comprehend,

that may well remain enigmatic, and

that at present lacks a reasonable therapeutic approach other than repeat the trick…

ORIGINAL ARTICLE *Infertility*

Endometrial scratching for subfertility: everyone's doing it

S. Lensen[1,*], L. Sadler[2], and C. Farquhar[1]

HR 2016

83% of clinicians recommend endometrial scratching prior to IVF

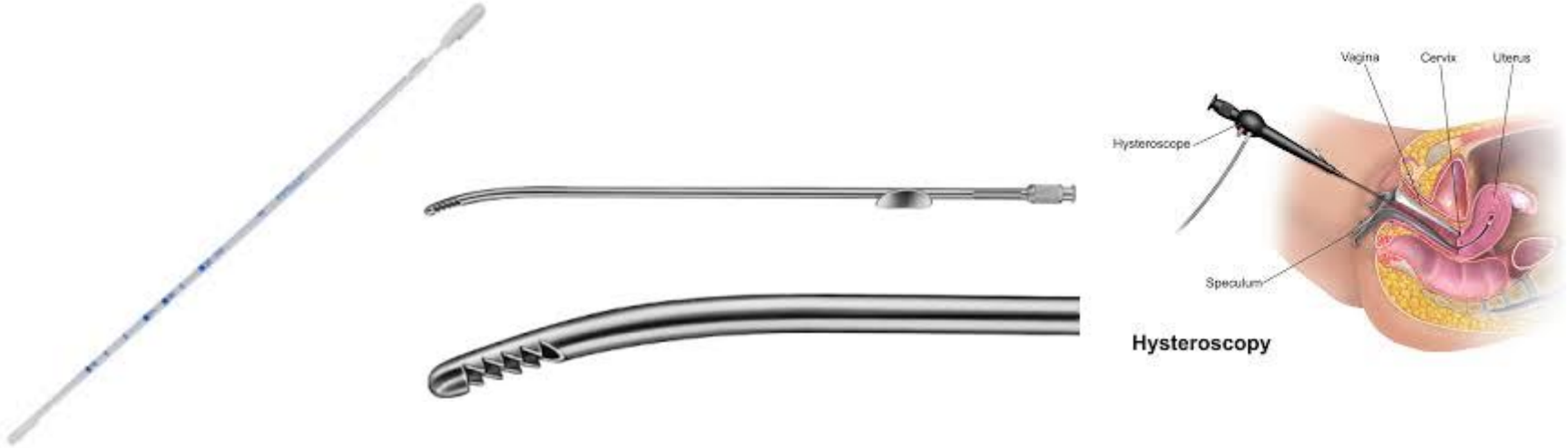
92% recommend endometrial scratching to women with RIF

What is endometrial scratching?

Endometrial scratching is:

Intentional mechanical injury to the endometrium

By endometrial biopsy catheter, curette or hysteroscopy



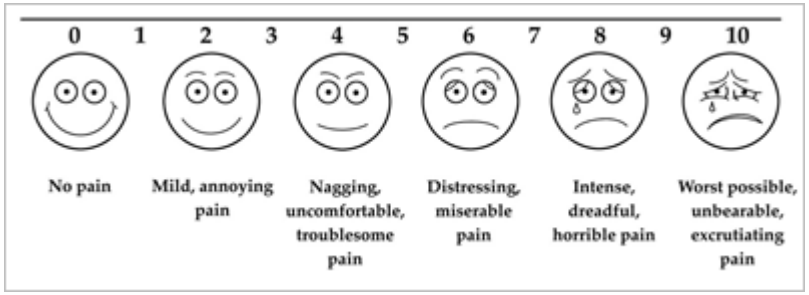
Usually in the luteal phase of the cycle BEFORE ovarian stimulation

With the objective to increase the chance of implantation

First described by Loeb in 1907 (rodent model)

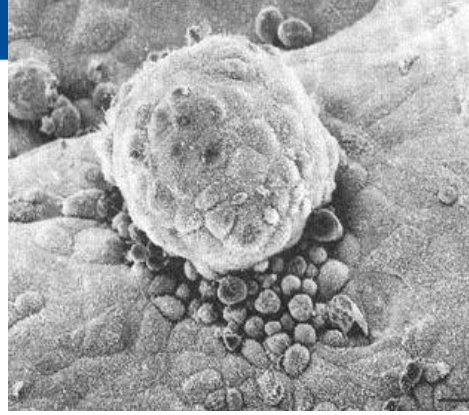
Importantly

Endometrial scratching:



- Is mildly painful (mean VAS 4 (range 0-7.5))
- Costs money (50-100 euro)
- Has a small risk of infection (<0.01%)

How would it increase implantation?



Hypotheses:

- decidualisation*
- 'wound healing' : aseptic inflammatory response (cytokines, growth factors, interleukins, macrophages) and vascular reaction (promoting neo-angiogenesis)
- delayed endometrial maturation (which is abnormally advanced during ovarian stimulation) and thereby improve embryo-endometrial synchrony in the next cycle

But the exact mechanisms are unclear and no data support these hypotheses

* However, unlike in rodents and other mammals, decidualization in humans is hormonally regulated, independent of any mechanical stimulus, and the presence or absence of conception.

Does it make scientific sense?

What happens in a normal cycle when a pregnancy fails to occur ?

Menstruation: regulated physiological breakdown & shedding

Endometrial proliferation: repair of an “injured/wounded” endometrium

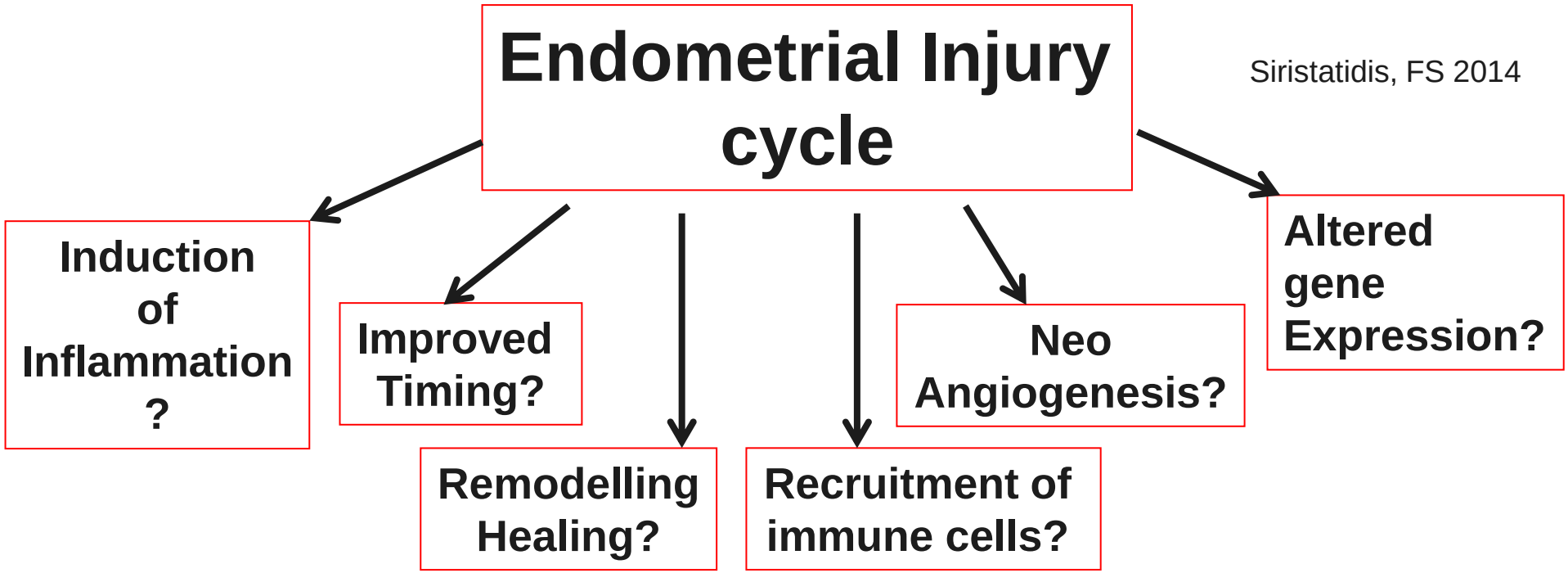
Through: hormone withdrawal, local tissue hypoxia, cyto/chemokine production and leukocyte traffic.

Most scratches are done in the preceding cycle: so what will it add?

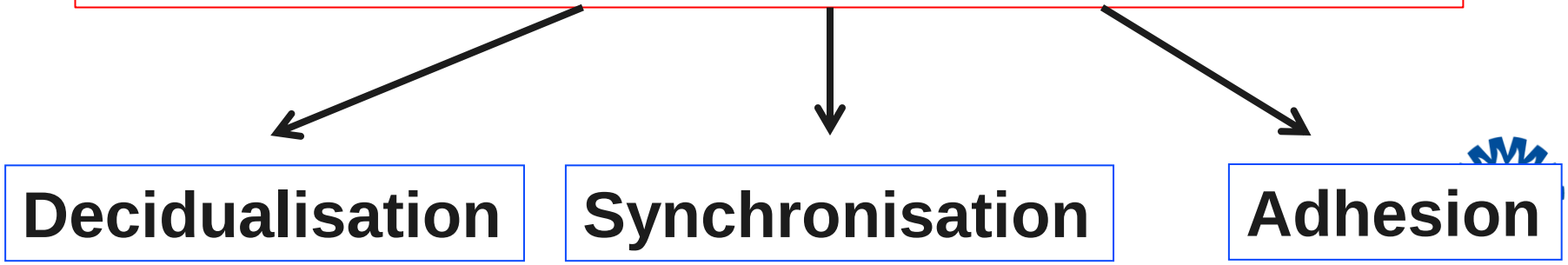
(as there is a menstruation between the scratch and the treatment cycle)

Endometrial Injury cycle

Siristatidis, FS 2014



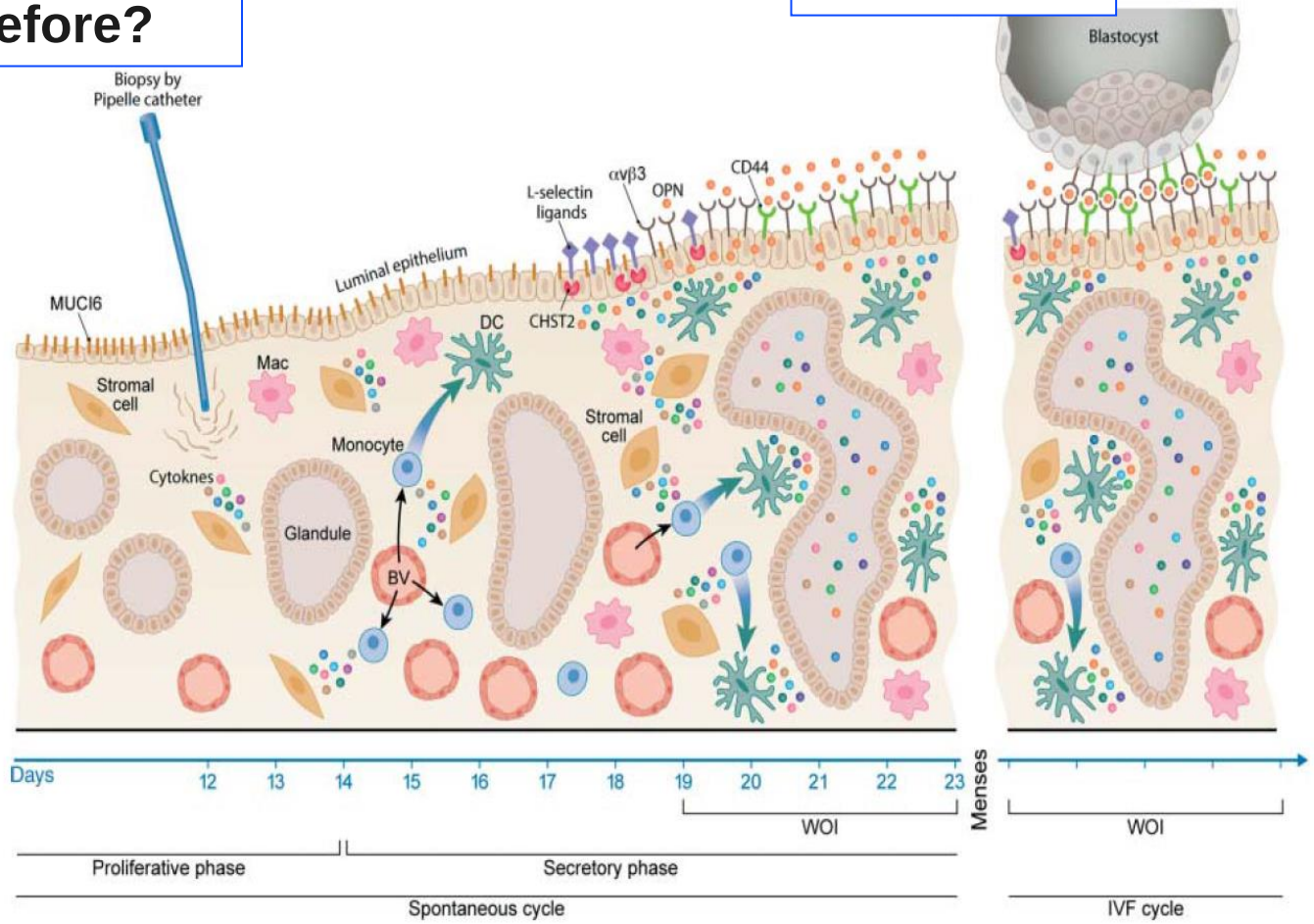
Effects Across Months? Influencing embryo transfer cycle?



Only if there is a carry over effect?

Start processes mentioned before?

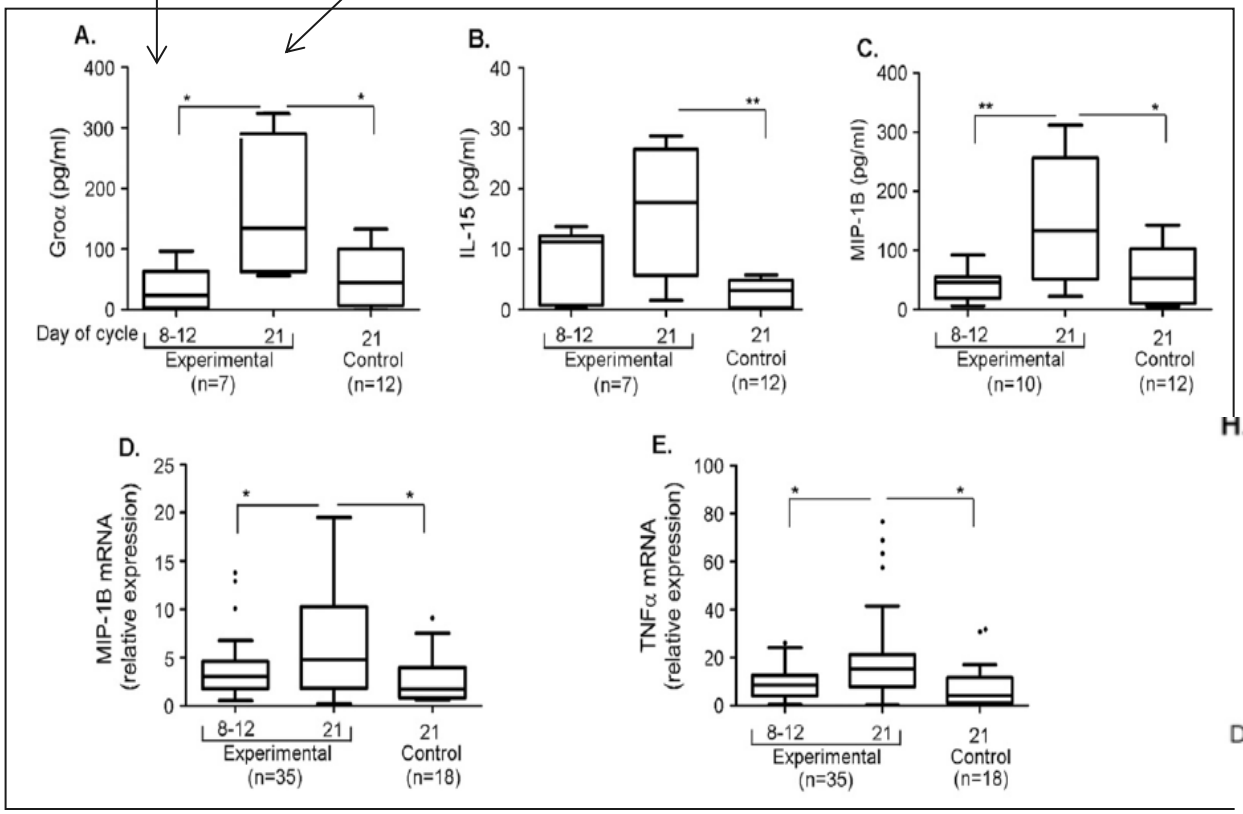
Carry Over Effect



Evidence from humans

First Biopsy CD 8-12

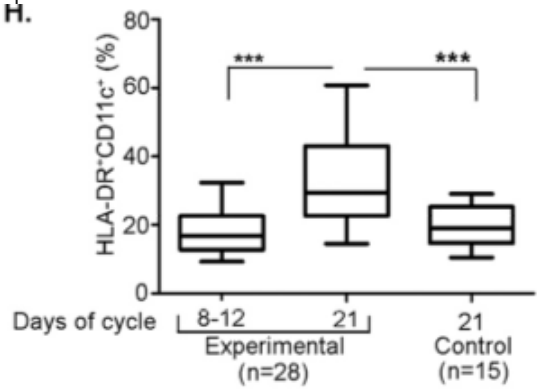
Second Biopsy CD21



Higher amount of:

- **Macrophages/dendritic cells (HLA-DR+CD11c+ cells)**
- **Growth-regulated oncogene-*α* (GRO-*α*),**
- **IL-15**
- **Macrophage inflammatory protein 1B (MIP-1B)**
- **TNF-*α***

Gnainsky, FS 2010



Which clinical trials have been done?

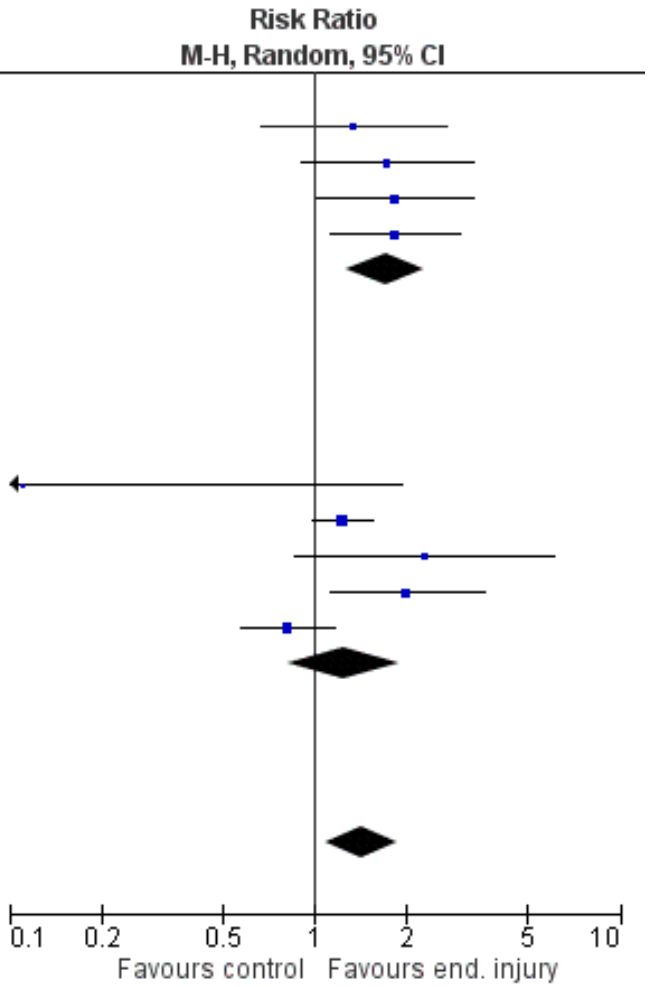
Meta-analysis was not possible due to significant clinical heterogeneity among the included studies. Patients' characteristics differed, as did the intervention used with endometrial injury being performed at different phases of the preceding menstrual cycle. Moreover, the effect of endometrial injury on live birth and clinical pregnancy rates were inconsistent among the included studies. In summary, there is currently insufficient evidence to support the use of endometrial injury in women with recurrent implantation failure undergoing assisted reproductive techniques while the procedure-associated complication rate has not been assessed. Clinical implementation should, thus, be deferred until robust evidence becomes available.

Review by Panagiotoupoulou et al 2015

Which clinical trials have been done?

LBR/OPR

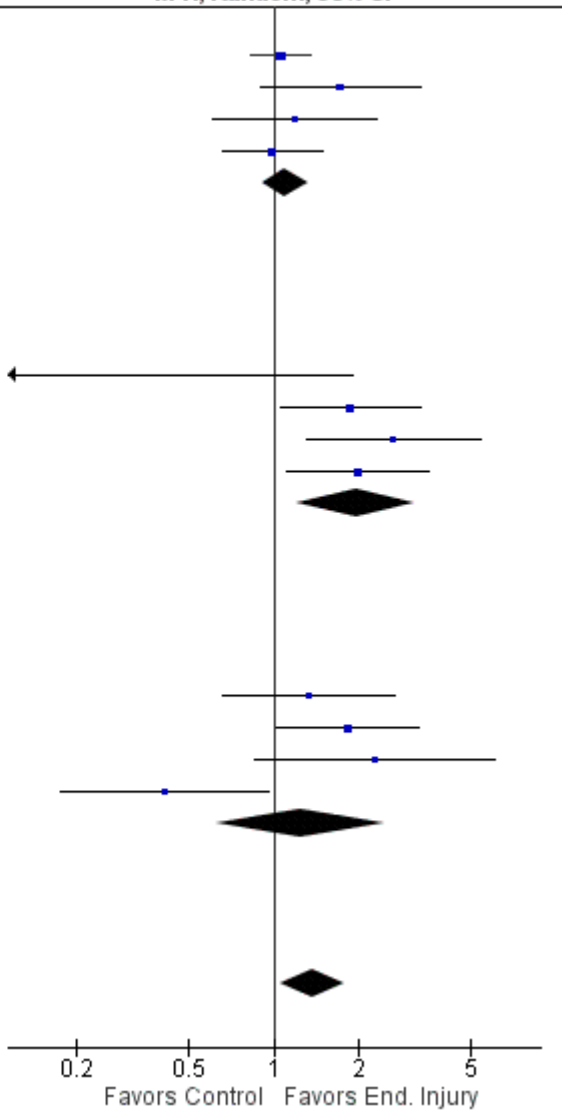
Study or Subgroup	End. Injury Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
1.1.1 No intrauterine manipulation in control group						
Aleyamma 2013	13	40	10	41	9.2%	1.33 [0.66, 2.68]
Guven 2014	19	62	11	62	10.0%	1.73 [0.90, 3.32]
Inal 2012	22	50	12	50	11.3%	1.83 [1.02, 3.29]
Nastri 2013	33	79	18	79	13.7%	1.83 [1.13, 2.97]
Subtotal (95% CI)		**231**		**232**	**44.2%**	**1.71 [1.28, 2.30]**
Total events	87		51			
Heterogeneity: Tau² = 0.00; Chi² = 0.62, df = 3 (P = 0.89); I² = 0%						
Test for overall effect: Z = 3.59 (P = 0.0003)						
1.1.2 Intrauterine manipulation in control group						
Baum 2012	0	18	4	18	0.9%	0.11 [0.01, 1.92]
Gibreel 2015	91	193	74	194	20.8%	1.24 [0.98, 1.56]
Narvekar 2010	11	49	5	51	5.7%	2.29 [0.86, 6.11]
Shohayeb 2012	28	105	14	105	11.4%	2.00 [1.12, 3.58]
Yeung 2014	39	150	48	150	17.1%	0.81 [0.57, 1.16]
Subtotal (95% CI)		**515**		**518**	**55.8%**	**1.25 [0.82, 1.90]**
Total events	169		145			
Heterogeneity: Tau² = 0.12; Chi² = 11.90, df = 4 (P = 0.02); I² = 66%						
Test for overall effect: Z = 1.02 (P = 0.31)						
Total (95% CI)		**746**		**750**	**100.0%**	**1.42 [1.08, 1.85]**
Total events	256		196			
Heterogeneity: Tau² = 0.08; Chi² = 17.10, df = 8 (P = 0.03); I² = 53%						
Test for overall effect: Z = 2.55 (P = 0.01)						
Test for subgroup differences: Chi² = 1.48, df = 1 (P = 0.22), I² = 32.5%						



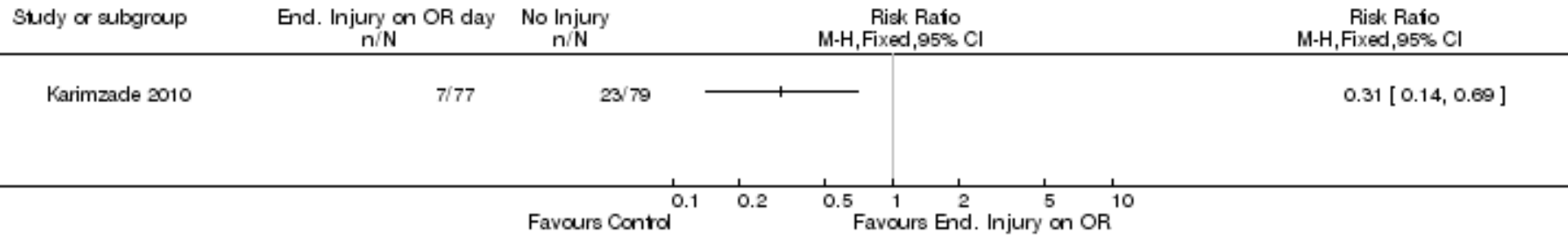
Review Nastri et al 2015

LBR/OPR

Study or Subgroup	End. Injury Events	End. Injury Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
1.2.1 ≤ 1 previous embryo transfer						
Gibreel 2015	68	129	60	121	15.0%	1.06 [0.83, 1.36]
Guven 2014	19	62	11	62	8.4%	1.73 [0.90, 3.32]
Nastri 2013	13	35	10	32	8.2%	1.19 [0.61, 2.32]
Yeung 2014	32	105	32	104	12.2%	0.99 [0.66, 1.49]
Subtotal (95% CI)		**331**		**319**	**43.8%**	**1.10 [0.91, 1.33]**
Total events	132		113			
Heterogeneity: Tau² = 0.00; Chi² = 2.23, df = 3 (P = 0.53); I² = 0%						
Test for overall effect: Z = 0.99 (P = 0.32)						
1.2.2 ≥ 2 previous embryo transfers						
Baum 2012	0	18	4	18	0.8%	0.11 [0.01, 1.92]
Gibreel 2015	23	64	14	73	9.6%	1.87 [1.06, 3.32]
Nastri 2013	20	44	8	47	7.7%	2.67 [1.31, 5.43]
Shohayeb 2012	28	105	14	105	9.4%	2.00 [1.12, 3.58]
Subtotal (95% CI)		**231**		**243**	**27.5%**	**1.96 [1.21, 3.16]**
Total events	71		40			
Heterogeneity: Tau² = 0.09; Chi² = 4.78, df = 3 (P = 0.19); I² = 37%						
Test for overall effect: Z = 2.74 (P = 0.006)						
1.2.3 Unselected women or unclear number of previous embryo transfers						
Aleyamma 2013	13	40	10	41	7.9%	1.33 [0.66, 2.68]
Inal 2012	22	50	12	50	9.4%	1.83 [1.02, 3.29]
Narvekar 2010	11	49	5	51	5.2%	2.29 [0.86, 6.11]
Yeung 2014	6	45	15	46	6.2%	0.41 [0.17, 0.96]
Subtotal (95% CI)		**184**		**188**	**28.6%**	**1.24 [0.63, 2.46]**
Total events	52		42			
Heterogeneity: Tau² = 0.33; Chi² = 9.72, df = 3 (P = 0.02); I² = 69%						
Test for overall effect: Z = 0.62 (P = 0.54)						
Total (95% CI)		**746**		**750**	**100.0%**	**1.37 [1.05, 1.79]**
Total events	255		195			
Heterogeneity: Tau² = 0.11; Chi² = 25.29, df = 11 (P = 0.008); I² = 57%						
Test for overall effect: Z = 2.34 (P = 0.02)						
Test for subgroup differences: Chi² = 4.78, df = 2 (P = 0.09), I² = 58.2%						



Review: Endometrial injury in women undergoing assisted reproductive techniques
Comparison: 2 Endometrial injury on the day of oocyte retrieval vs control
Outcome: 1 Live birth or ongoing pregnancy per randomly assigned woman



Author's conclusions: endometrial scratching

- between day 7 of the previous cycle and day 7 of the ET cycle is associated ↑ live birth rates in women with 2 or more previous embryo transfers (moderate-quality evidence).

- on the day of oocyte retrieval is associated with ↓ ongoing pregnancy rates .

Although current evidence suggests some benefit of endometrial injury, we need evidence from ***well-designed trials that do not cause endometrial damage in the control group, stratify the results for women with and without RIF and report live birth.***

Clinical heterogeneity

Even with new studies clinical heterogeneity will remain

This could possibly be solved by performing an independent patient data (IPD) meta-analysis

Van Hoogenhuijze registered in PROSPERO (International prospective register of systematic reviews) 2017

First meeting ESHRE 2017

The SCRaTCH trial

Design:

National, multicentre (academic and non-academic) randomized, non-blinded, controlled trial with a cost-efficacy analysis

Nested cohort study in 6 clinics in which endometrial biopsies are banked

consortium 3.0

ZonMw

Study population

Women (18-43 years of age) starting their 2nd IVF/ICSI cycle with implantation failure

(defined as the absence of an ongoing pregnancy occurring after a full first ART cycle with ≥1 embryo transfer)

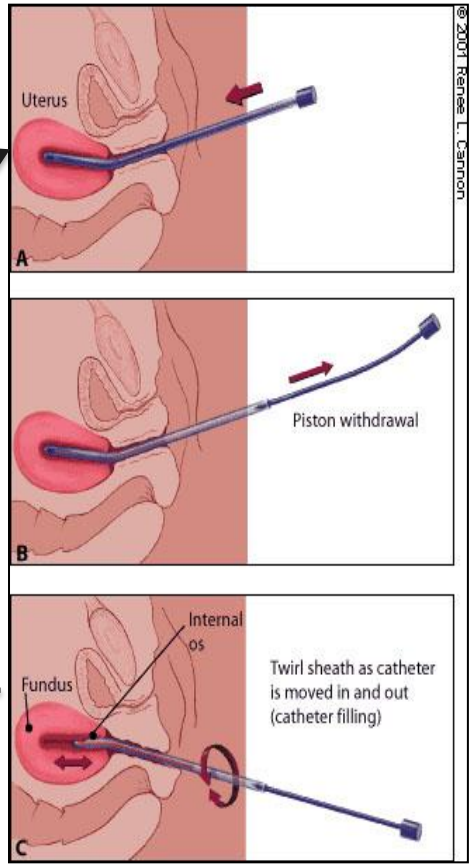
1st full ART cycle → Ongoing pregnancy

Failed implantation after at least 1 embryo transered (fresh or frozen)

Randomisation SCRaTCH study

No Scratch

Scratch



2nd fresh ART treatment cycle (Primary outcome: live birth after 2nd fresh ART cycle)

Data from all cycles within **12 months** after randomisation will be included in the study

Sample size calculation

9% expected difference in live birth rate after the 2nd full ART cycle (39% versus 30%)

450 women per study arm for 80% power ($\alpha = 0.05$) to detect such a difference

First inclusion January 2016

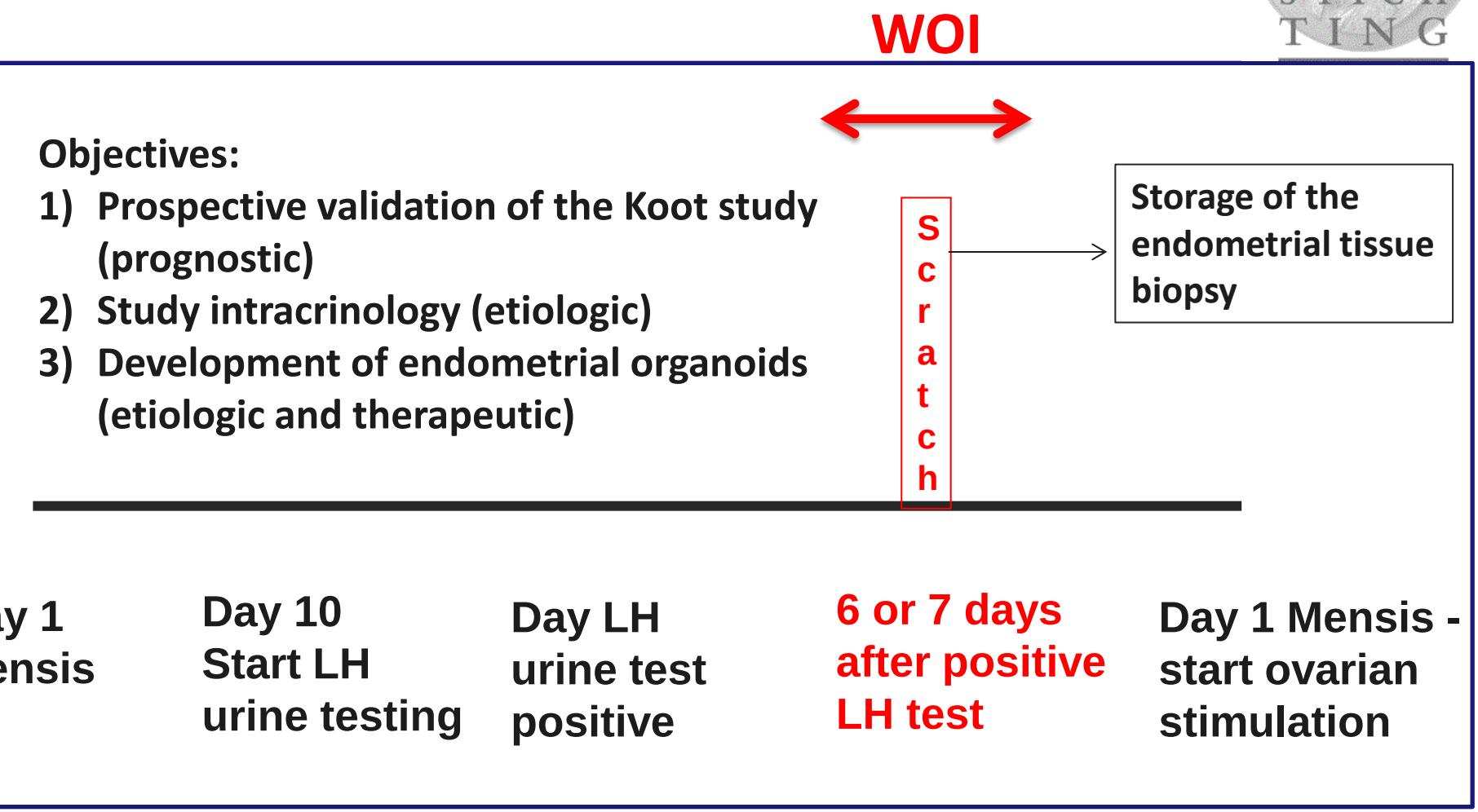
Now 600+ inclusions

Expected completion summer of 2018

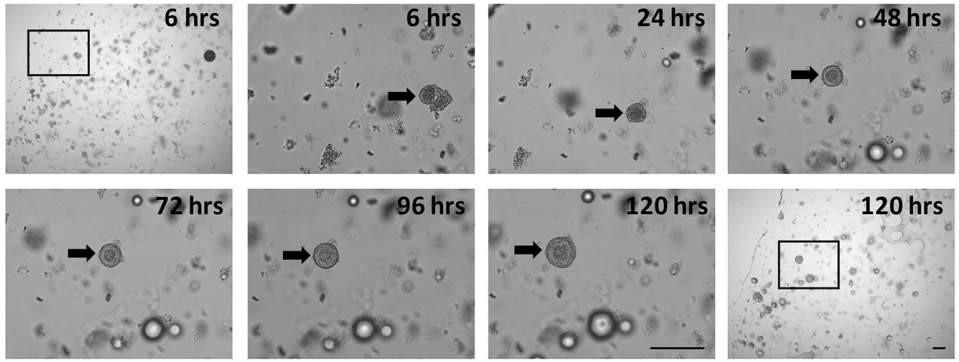
Participating clinics

- Academisch Medisch Centrum
- Albert Schweitzer ziekenhuis
- Alrijne ziekenhuis
- Amphia ziekenhuis
- Catharina ziekenhuis
- Deventer ziekenhuis
- Diakonessenhuis Utrecht
- Elisabeth-Twee Steden ziekenhuis
- Erasmus Medisch Centrum
- Fertiliteitskliniek Twente
- Gelderse Vallei
- Gelre ziekenhuis Apeldoorn
- Groene Hart ziekenhuis
- Isala klinieken
- Jeroen Bosch ziekenhuis
- Leids Universitair Medisch Centrum
- Maasstad ziekenhuis
- Maastricht Universitair Medisch Centrum
- Maxima Medisch Centrum
- Meander Medisch Centrum
- Medisch Centrum Kinderwens
- Nij Geertgen Ziekenhuis
- Noordwest Ziekenhuisgroep, locatie Gemini
- Onze Lieve Vrouwe Gasthuis, locatie West
- Radboud Medisch Centrum
- Scheper ziekenhuis
- Sint Antonius ziekenhuis
- Sint Franciscus Gasthuis
- Ter Gooi ziekenhuis
- Universitair Medisch Centrum Groningen
- Vrije Universiteit Medisch Centrum
- Wilhelmina Ziekenhuis Assen

SCRaTCH-2 nested study



Organoids



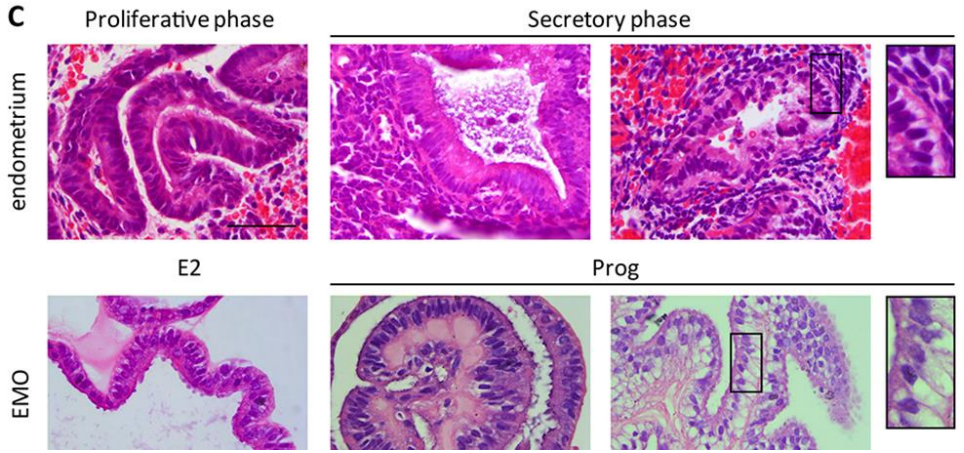
Self-forming 3D reconstructions of an organ's epithelium,

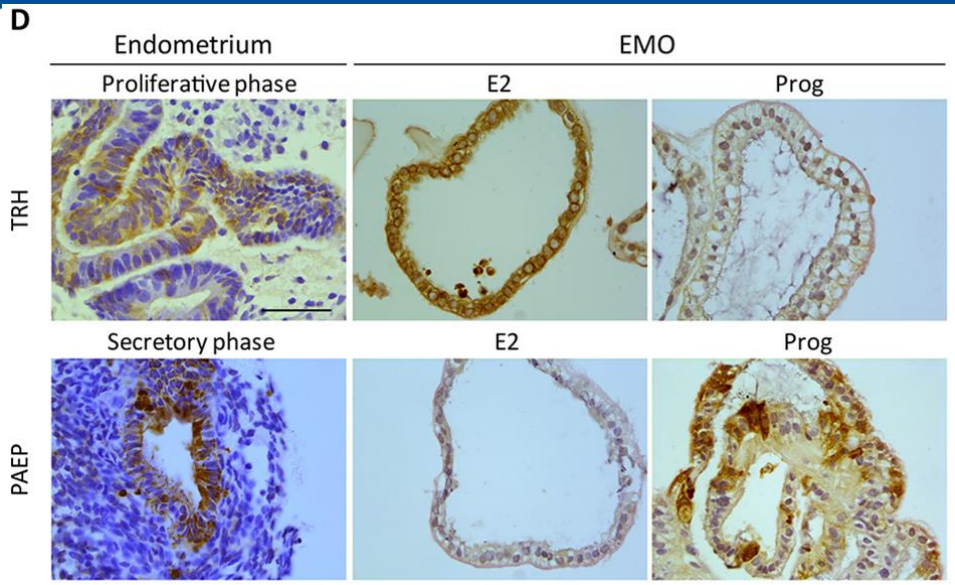
High expansion capacity

Retention of phenotypical and functional properties

Overcoming the limited availability or expandability of primary human tissue

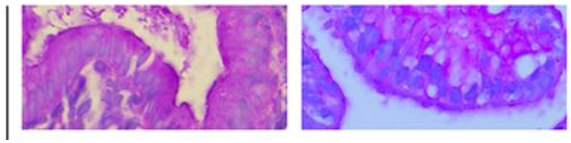
Boretto et al 2017



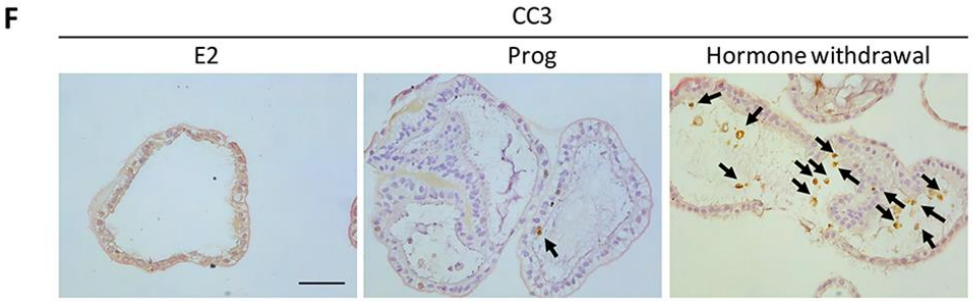


Thyrotropin releasing hormone expressed in proliferative phase

Progestagen-associated endometrial protein expressed in secretory phase



Mucin observed in prog treated EMO



Apoptotic cells visible after hormone withdrawal

Boretto et al 2017

ORIGINAL ARTICLE *Infertility*

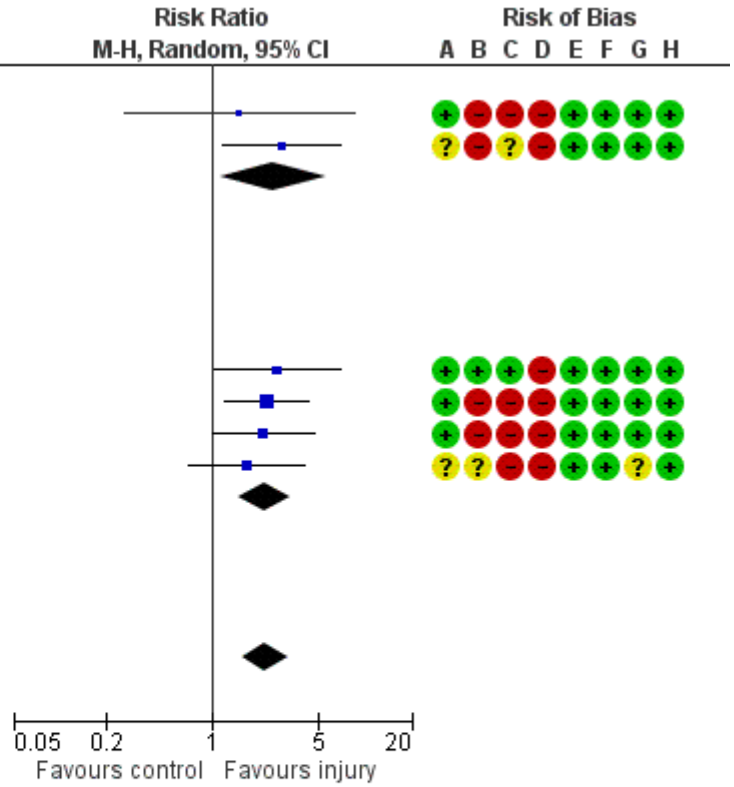
Endometrial scratching for subfertility: everyone's doing it

S. Lensen[1,*], L. Sadler[2], and C. Farquhar[1]

Additionally, 4% of clinicians recommend endometrial scratching to women undergoing intrauterine insemination or trying to conceive naturally.

Which clinical trials have been done?

Study or Subgroup	Endometrial injury Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
1.1.1 Live birth						
Mahey 2015 (1)	3	43	2	43	4.1%	1.50 [0.26, 8.53]
Parsanezhad 2013 (2)	17	117	6	117	15.3%	2.83 [1.16, 6.93]
Subtotal (95% CI)		**160**		**160**	**19.4%**	**2.48 [1.12, 5.49]**
Total events	20		8			
Heterogeneity: Tau² = 0.00; Chi² = 0.41, df = 1 (P = 0.52); I² = 0%						
Test for overall effect: Z = 2.24 (P = 0.03)						
1.1.2 Ongoing pregnancy						
Gibreel 2013 (3)	14	54	5	51	13.7%	2.64 [1.03, 6.82]
Maged 2016 (4)	25	77	11	77	30.4%	2.27 [1.20, 4.29]
Wadhwa 2015 (5)	30	150	7	75	20.4%	2.14 [0.99, 4.65]
Zarei 2014 (6)	12	74	7	72	16.1%	1.67 [0.70, 4.00]
Subtotal (95% CI)		**355**		**275**	**80.6%**	**2.16 [1.46, 3.19]**
Total events	81		30			
Heterogeneity: Tau² = 0.00; Chi² = 0.54, df = 3 (P = 0.91); I² = 0%						
Test for overall effect: Z = 3.87 (P = 0.0001)						
Total (95% CI)		**515**		**435**	**100.0%**	**2.22 [1.56, 3.15]**
Total events	101		38			
Heterogeneity: Tau² = 0.00; Chi² = 1.04, df = 5 (P = 0.96); I² = 0%						
Test for overall effect: Z = 4.46 (P < 0.00001)						
Test for subgroup differences: Chi² = 0.09, df = 1 (P = 0.76), I² = 0%						



Footnotes

(1) IUI
(2) Intercourse
(3) Intercourse
(4) IUI
(5) IUI, Intervention groups added together
(6) IUI

Risk of bias legend

(**A**) Random sequence generation (selection bias)
(**B**) Allocation concealment (selection bias)
(**C**) Blinding of participants (performance bias)
(**D**) Blinding of personnel (performance bias)
(**E**) Blinding of outcome assessment (detection bias)
(**F**) Incomplete outcome data (attrition bias)
(**G**) Selective reporting (reporting bias)
(**H**) Other bias

Lensen et al Cochrane 2016

GRADE quality low to very low

The SCRaTCH-OFO trial

SCRATCH-OFO

Similar design to IVF trial with some important differences

- Couples with unexplained subfertility and a good prognosis of spontaneous conception according to Hunault

- In principle 6 months of timed intercourse ('expectant management') before ART is started

Sample size 792 women

First inclusions UMCU November 2017 (n=5)

consortium 2.0

ZonMw

Hunault

Freya

To be used in women < 38 years of age diagnosed with unexplained subfertility

Is er een Samenlevingstest gedaan ? Nee Go

Leeftijd vrouw	20 Jaar 0 Maanden
Duur onvruchtbaarheid in jaren	1 Jaar 0 Maanden
Bent u al eerder spontaan zwanger geweest (huidige of vorige relatie) ?	Nee
Verwezen door :	Huisarts of zelf naar Gynaecoloog gegaan
Hoeveel procent van het zaad is goed beweeglijk ?	10

Bereken

Deze prognose is in de periode 2002-2004 in 38 ziekenhuizen gebruikt in het OFO project waaruit is gebleken dat de modellen werken.

Chance of spontaneous conception < 12 months

If chance > 30%: advice to continue trying to conceive naturally for 6-12 months

UMC Utrecht

To SCRaTCH or not to scratch

that's the question

<u>YES to scratch, but only in trials:</u> because scratching

- **costs money**
- **produces pain**
- **gives a small, but important, risk of infection**

SCRATCH-OFO

Quote Santamaria et al 2016

"Would clinicians and patients accept the performance of an intervention named 'retinal scratching' without any proven benefit for vision?

Clearly, the answer is no.

Thus, we must consider the importance of information with respect to endometrial scratching.

It is easier to scratch than to think, but, please, think before you scratch."

Thank you to

All clinics and women participating in the SCRaTCH trials

Frank Broekmans

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SCRaTCH-2 group:
Nils Lambalk
Ben Cohlen
Jan Peter de Bruijn
Ron van Golde
Gijs Teklenburg

Kathrin Fleisher

Organoid collaborators Leuven:
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DE SNOO VAN 'T HOOGER HUIJS STICHTING

Intracrinology collaborators MUMC:
Ron van Golde
Andreja Romano
Linda Brentjens

Endometrial gene expression collaborators:
Nick Macklon Copenhagen
Mette Nyegaard Arhus
Endometrial gene expression financers:
Merck

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UMC Utrecht