

WHAT IS NEW IN RIF?

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Probability of failed implantation- good quality cleavage stage

Number of embryos transferred	Probability of failed implantation
1	70%
2	49%
3	34%
4	24%
5	17%
6	12%

Probability of failed implantation- poor quality cleavage stage

Number of embryos transferred	Probability of failed implantation
1	90%
2	73%
3	66%
4	59%
5	53%
6	48%

Probability of failed implantation- good quality blastocyst stage

Number of embryos transferred	Probability of failed implantation
1	60%
2	36%
3	22%
4	13%

How do I define RIF?

- Failed implantation beyond chance occurrence

Definition of RIF

- Number of cycles
- Number of embryos
- Cleavage vs blastocyst embryos
- Fresh vs frozen embryos

Failure to achieve a clinical pregnancy after transfer of at least 4 good-quality embryos in a minimum of three fresh or frozen cycles in a woman under the age of 40 years (Coughlan et al. [RBM Online 2014](#))

Pragmatic classification of RIF

- Expected-recurrent IVF failure
- Unexpected-recurrent implantation failure

Expected RIF

- Advanced maternal age
- Reduced ovarian reserve
- Poor quality embryos
- Atrophic endometrium

*Do we
need
to
investigate
further?*

Expected RIF-anticipated implantation rate $\leq 10\%$

Cycle number	Number pregnant out of 100
0	0
1	10
2	19
3	24
4	31

App 70% of patients not pregnant after 4 treatment cycle

Poor quality embryos vs embryos with diminished potential to implant

- Poor oocyte quality-poor embryo development and fertilization
 - ▣ Advanced maternal age
 - ▣ Poor ovarian reserve
 - ▣ Abnormal cumulus cell gene expression profile
- Sperm DNA damage-poor embryo development and fertilization
 - ▣ Smoking
 - ▣ Genital tract infections
 - ▣ Chemo-radiotherapy
- Genetic factors
 - ▣ Translocations (x2.5 in the RIF population)
 - ▣ Epigenetic factors

Unexpected RIF

- Young age
- Adequate ovarian reserve
- Good quality embryos
- No pelvic pathology on routine scan

Investigate

Unexpected RIF-anticipated implantation rate $\geq 30\%$

Cycle number	Number pregnant out of 100
0	0
1	30
2	51
3	65
4	75

App 25% of patients not pregnant after 4 treatment cycle

Evaluation of RIF

- Imaging of the pelvis
 - Uterus
 - Ovaries
 - Tubes
- Hysteroscopy
- Evaluation of possible immunological problems
- Genetic factors



IMAGING



Detailed Imaging

- Transvaginal high resolution US+3D-intracavitary and intramural lesions
- HSG-synechia, hydrosalpinx
- MRI-adenomyosis, fibroids

Fibroids and reproductive outcomes: a systematic literature review from conception to delivery

Peter C. Klatsky, MD; Nam D. Tran, MD, PhD; Aaron B. Caughey, MD, PhD; Victor Y. Fujimoto, MD

TABLE 1
Submucosal fibroids

	Study design; number of patients	Fibroid diameter: mean (range)	Implantation rate ^a		Clinical pregnancy rate ^b		Spontaneous abortion rate ^c	
			Fibroids	No fibroids	Fibroids	No fibroids	Fibroids	No fibroids
Farhi et al ^{7d}	Retrospective cohort. 18 IM fibroid patients, 50 controls	(<70 mm)	2.7% 5/179	9.8% 35/357	9.7% 5/55	25.2% 32/127	40% 2/5	25% 8/32
Eldar-Geva et al ^{9d}	Retrospective cohort. 6 study patients, 249 controls.	45 mm ± 26	4.3% 1/23	12.3% 94/763	10% 1/10	30.8% 98/318	0% 0/1	16.3% 16/98
Cassini et al ¹⁴ (SM)	Prospective, observational. Spontaneous conceptions, following timed intercourse.	(<40 mm)			21.4% 9/42	40.4% 21/52	55.6% 5/9	42.9% 9/21
Cumulative rates			3.0% 6/202 OR 0.39 (0.24-0.65)	11.5% 129/1120	14% 15/107 OR 0.44 (0.28-0.70)	30.4% 151/497	46.7% 7/15 OR: 3.85 (1.12-13.27)	21.9% 33/151

IM, intramuscular; OR, odds ratio; SM, submucosal.

^a No. of sacs per no. of embryos transferred.

^b No. of cycles with a gestational sac or living embryo per transfer.

^c Clinical miscarriage after documented clinical pregnancy.

^d Included repeat cycles in the same patient.

Klatsky. *Fibroids and reproductive outcomes. Am J Obstet Gynecol* 2008.

The effect of non-cavity-distorting fibroids on IVF outcome

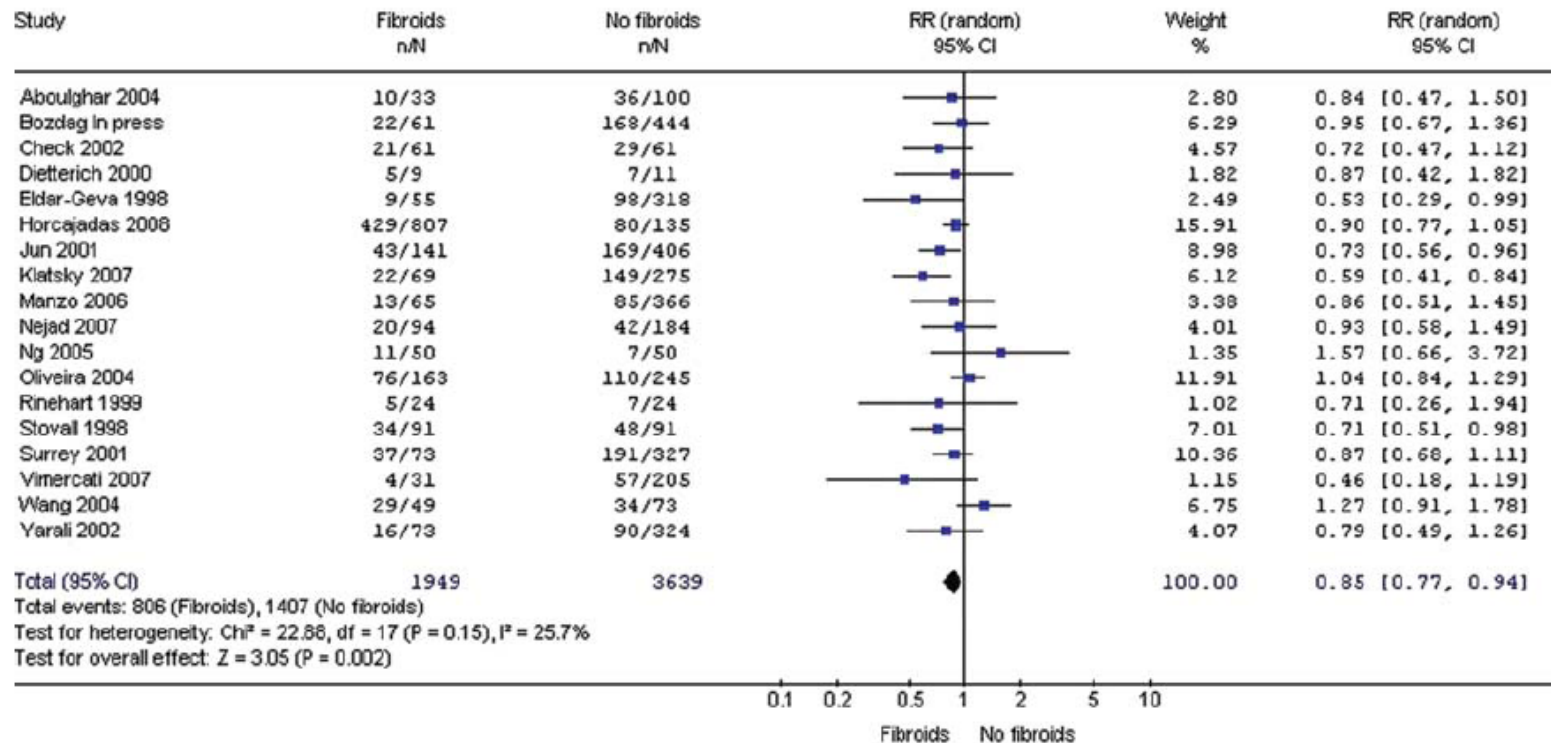


Figure 7 Forest plot of studies of non-cavity-distorting intramural fibroids versus no fibroids in women undergoing IVF treatment for outcome of clinical PRs.

From Sunkara et al. Hum Reprod 2010

Hydrosalpinx



Effect of untreated hydrosalpinx

Table VI. Meta-analysis

Outcome criteria	Group with hydrosalpinx (%)	Group without hydrosalpinx (%)	Odds ratio	Confidence interval
Pregnancy rate	19.67	31.2	0.64	0.56–0.74 ^a
Implantation rate	8.53	13.68	0.63	0.55–0.72 ^a
Delivery rate	13.4	23.44	0.58	0.49–0.69 ^a
Early pregnancy loss rate	43.65	31.11	1.72	1.34–2.20 ^a

^aOdds ratio significantly different from 1 ($P < 0.05$).

Effect of removal of hydrosalpinx

- Odds of pregnancy = 1.75 (1.1-2.9)
- Odds of ongoing pregnancy = 2.13 (1.2-3.7)
- Embryo implantation = 1.34 (0.9-2.1)
- Ectopic pregnancy=0.42 (0.1-2.1)
- Miscarriage=0.49 (0.2-1.5)

Cochrane review
Johnson et al. 2002

Endometriosis

- Only 1 study showed that surgical treatment of endometriosis may be beneficial in women with RIF
 - ▣ Retrospective
 - ▣ 23 patients
 - ▣ Almost half of the patients conceived spontaneously after laparoscopy

Adenomyosis

- Recently associated with RIF
- Only 2 prospective studies
- Universal agreement on diagnosis ??
 - ▣ USG
 - ▣ Doppler
 - ▣ MRI

Adenomyosis and outcome of IVF-clinical pregnancy rates

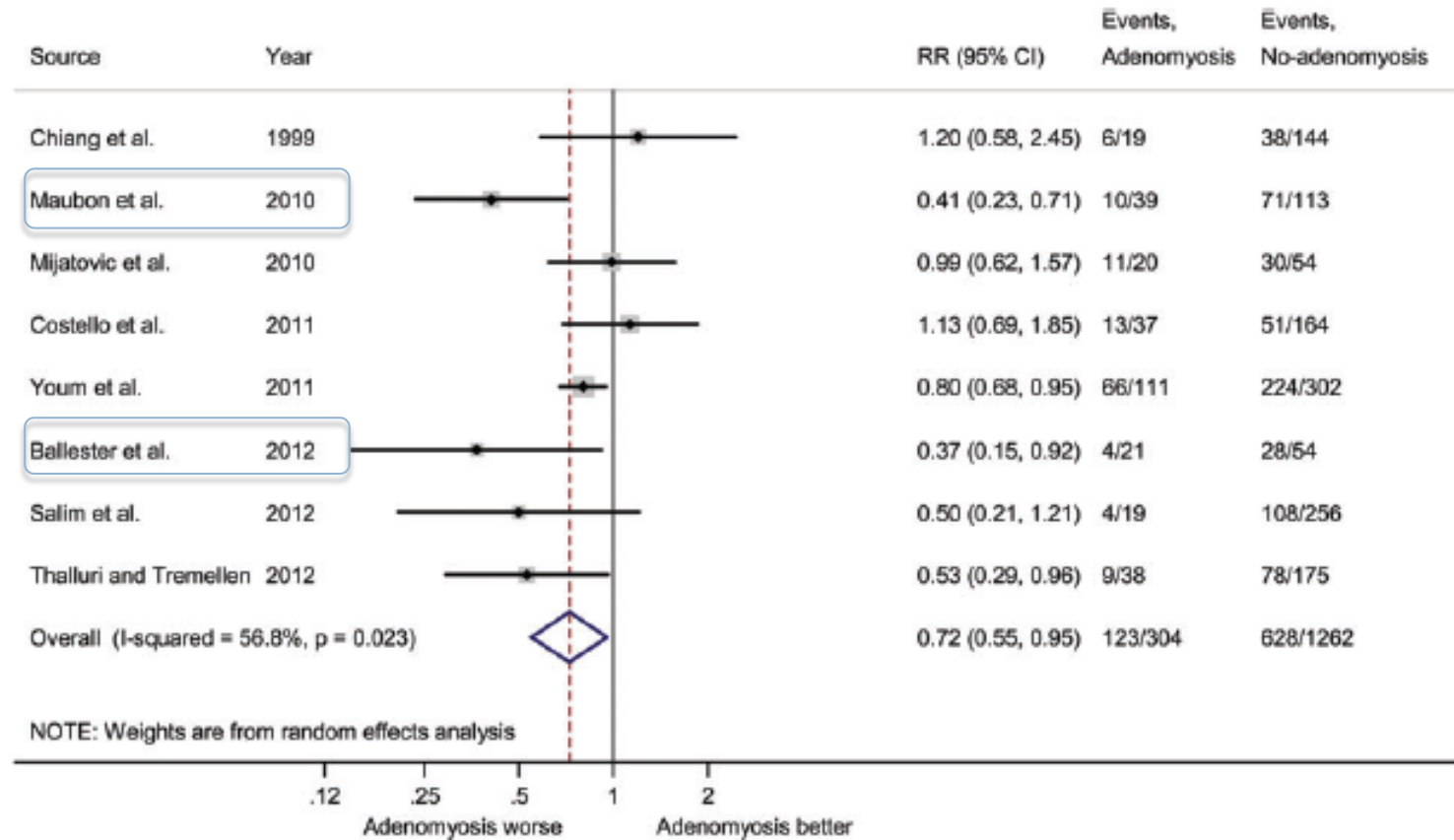


Figure 2 Forest plot showing individual and combined effect size estimates and 95% confidence intervals (CIs) in studies that evaluated the likelihood of clinical pregnancy in infertile women with or without adenomyosis undergoing IVF/ICSI. Horizontal lines indicate 95% CIs; boxes show the study-specific weight; diamond represents combined effect size; dashed line indicates the overall estimate.

Adenomyosis and outcome of IVF-miscarriage rates

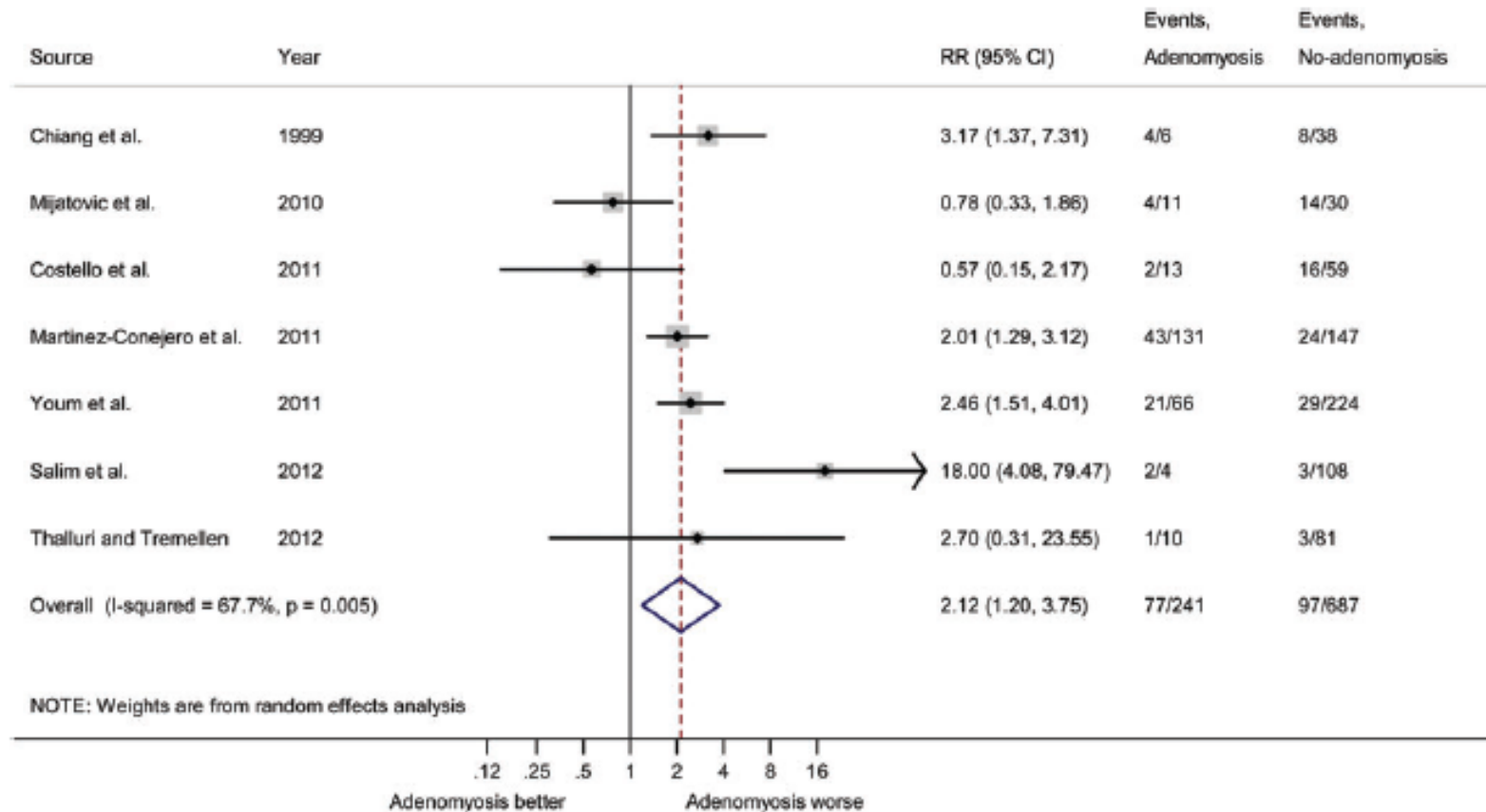


Figure 3 Forest plot showing individual and combined effect size estimates and 95% confidence intervals (CIs) in studies that evaluated the risk of miscarriage in clinical pregnancies obtained at IVF/ICSI in women with or without adenomyosis. Horizontal lines indicate 95% CIs; boxes show the study-specific weight; diamond represents combined effect size; dashed line indicates the overall estimate.



HYSTEROSCOPY



Hysteroscopy-emerging role in IVF and RIF

- Prior to the first IVF cycle
- After implantation failure/s

Outpatient hysteroscopy

- After 2 or more failed cycles 15-40% of patients will have an intra-cavitary lesion
(Olivera et al., 2003; Levi Setti, 2004; Urman, 2005)
- Polyps
- Adhesions
- Small fibroids
- Arcuate/subseptate uterus
- Endometritis
- Hyperplasia

Problems associated with hysteroscopic cavity evaluation

- Inter/intra-observer agreement regarding both normal and abnormal findings
- The significance of abnormal findings is not clear
- Whether treatment improves implantation rates is unknown

Observer agreement in the evaluation of the uterine cavity prior to IVF

Table II Findings of the hysteroscopy performer at real-time hysteroscopy.

Findings	Prevalence	(%)
Normal cavity	94	87.9
Abnormal cavity	13 ^a	12.1
Polyp	12	11.2
Myoma	1	0.9
Adhesion	0	0.0
Septa	2	1.9
Total	107 ^a	100

^aIn two cases more than one abnormality was detected.

Table III Level of overall perfect observer agreement expressed in κ coefficients/ICC^a.

Finding	Intraobserver agreement (%)	κ	95% CI
Normal cavity	93.5	0.707	0.517–0.897
Polyp	93.5	0.683	0.463–0.903
Myoma ^b	99.1	0.662	0.043–1.281
Adhesions ^b	99.1	–0.009	–0.198–0.180
Septum ^b	100	1.000	^c
Finding	Interobserver agreement (%)	ICC ^a	95% CI
Normal cavity	77.6	0.491	0.378–0.598
Polyp	83.2	0.511	0.399–0.616
Myoma ^b	95.6	0.281	0.161–0.406
Adhesions ^b	96.3	–0.018	–0.116–0.100
Septum ^b	93.8	0.475	0.360–0.584

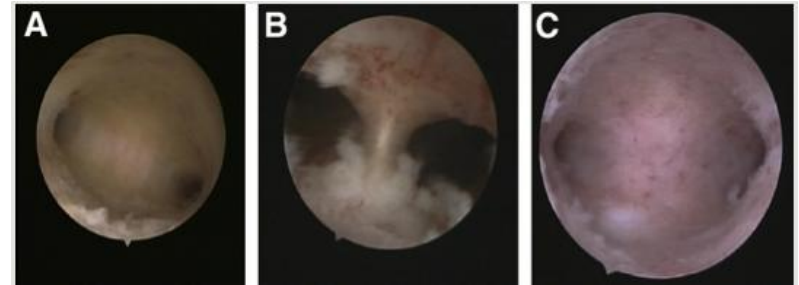
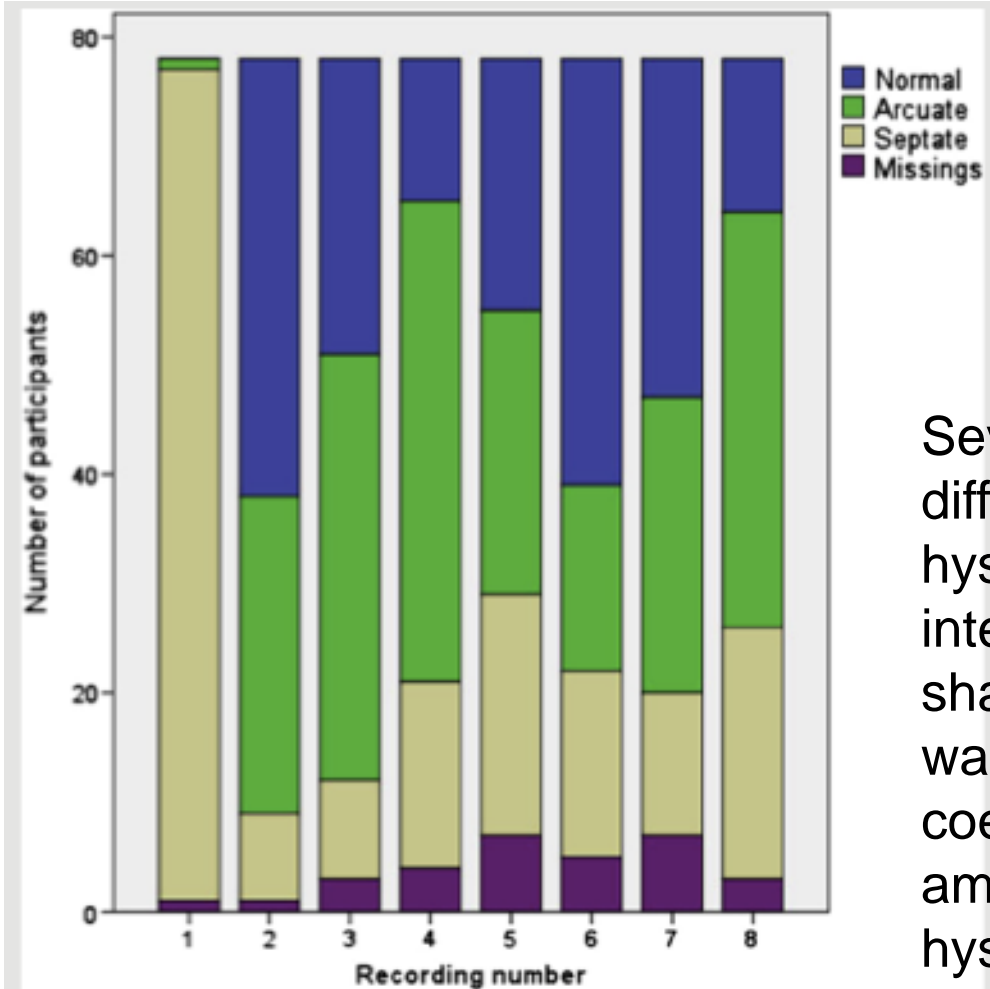
^aICC, intraclass correlation coefficient (equivalent of the overall weighted κ) (Fleiss and Cohen, 1973).

^bThe discrepancy between the perfect agreement and mean κ value is caused by the low prevalence of these abnormalities (Feinstein and Cicchetti, 1990).

^cImpossible to compute with ordinary statistics, as also used by SPSS version 15.1.

From Kasius et al. Hum Reprod 2011

Agreement on the diagnosis of septate uterus



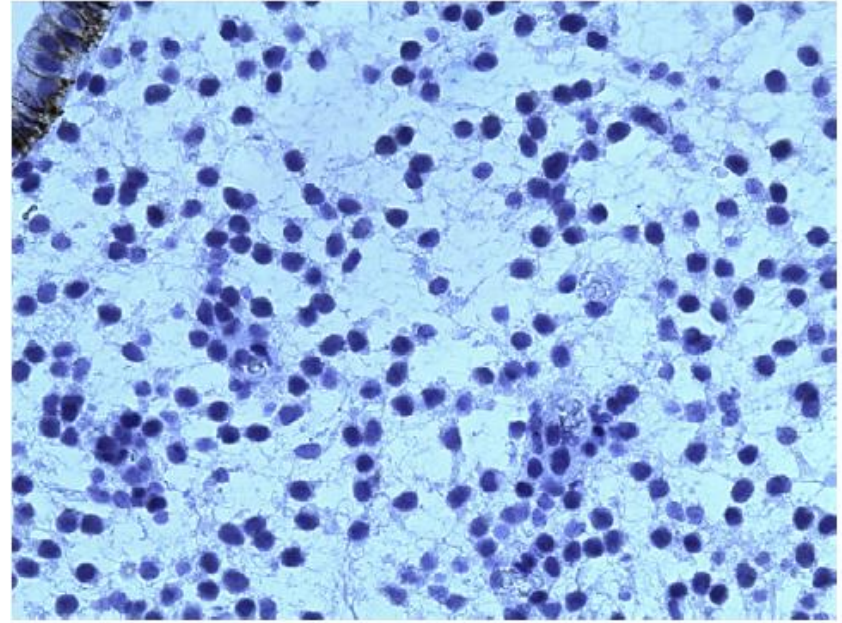
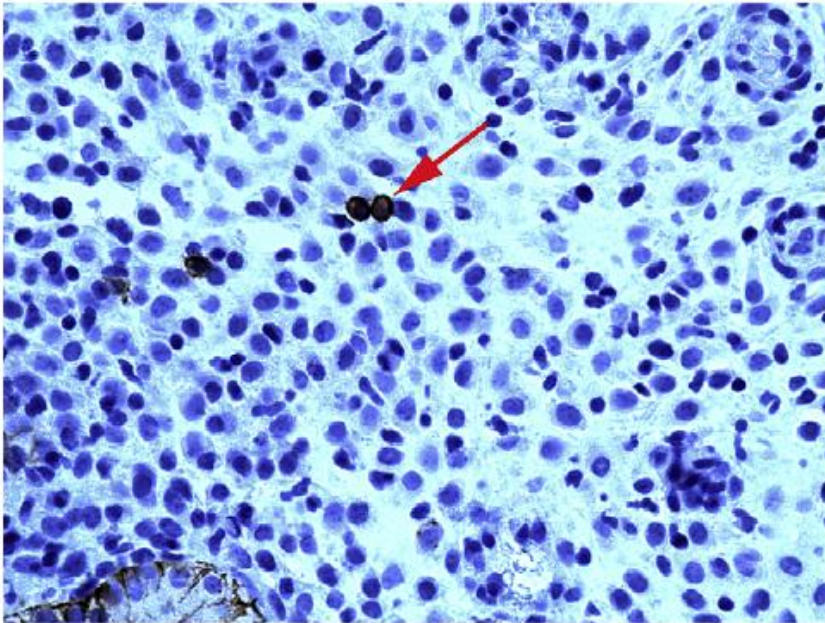
Seventy-eight observers from 24 different countries assessed 8 hysteroscopy recordings. The interobserver agreement on uterine shape variations septate and arcuate was fair (intraclass correlation coefficient $\frac{1}{4}$ 0.27). The agreement among international experts on the hysteroscopic diagnosis of the septate uterus was found to be poor.

Opinion on uterine shape.

Smit. Hysteroscopic agreement on septate uterus. *Fertil Steril* 2013.

From Smit et al. *Fertil Steril* 2013

Chronic endometritis



- Does it really exist?
- Is it a distinct clinical entity or an incidental finding?
- Is there a proven specific treatment for the condition?

Chronic endometritis is a significant finding in patients with RIF and should be treated

TABLE 1

Patient data: mean ± SD.

	Group 1 (n = 10)	Group 2 (n = 23)	Group 3 (n = 485)	P value
Age (years)	34.5 ± 3.27	34.69 ± 3.34	36.0 ± 0.17	NS
Number of previous failed cycles (n)	3.0 ± 1.63	2.73 ± 0.91	2.9 ± 0.03	NS
Number of mature oocytes retrieved (n)	14.7 ± 3.2	12.7 ± 1.3	10.4 ± 0.2	NS
Fertilization rate %	72.0 ± 0.12	63.9 ± 0.05	67.1 ± 0.01	NS
Good-quality ET (n)	2.1 ± 0.87	1.69 ± 1.01	1.75 ± 0.55	NS
ET (n)	2.6 ± 0.96	2.39 ± 1.19	3.1 ± 0.04	.001
Implantation rate % (n)	11.5 (3/26)	32.7 (18/55)	20.3 (301/1485)	.0024
Clinical pregnancy rate % (n)	20 (2/10)	52.1 (12/23)	40.6 (197/485)	NS
Clinical loss Rate % (n)	10 (1/10)	0 (0/23)	6.2 (30/485)	NS
Ongoing pregnancy rate % (n)	10 (1/10)	52.1 (12/23)	34.4 (167/485)	NS

Johnston-MacAnanny. Chronic endometritis in RIF. Fertil Steril 2010.

Group 1-Patients with chronic endometritis (confirmed by immunohistochemistry)

Group 2-Patients who did not have chronic endometritis

Group 3-Patients who did not undergo endometrial biopsy

From Johnston-McAnanny et al. Fertil Steril

Chronic endometritis has no effect on IVF outcome!

TABLE 2

IVF/ICSI results of the patients with chronic endometritis (case subjects) compared with control subjects.

Variables	Case (n = 17)	Control (n = 68)	Significance
No. of started cycles ^a	2.5 ± 2.1	2.5 ± 1.8	.91 ^b
Fresh cycles	2.2 ± 1.9	1.8 ± 1.2	.33 ^b
Cryocycles	0.3 ± 0.8	0.7 ± 1.3	.09 ^b
No. of embryo transfers	2.0 ± 1.6	2.2 ± 1.7	.70 ^b
No. of embryos transferred per cycle	1.4 ± 0.5	1.4 ± 0.4	.45 ^b
Live birth	13 (76%)	37 (54%)	.11 ^c

Note: The control group consisted of a randomly selected sample of patients without endometritis, matched for the research hospital and day of menstrual cycle on which the biopsy was performed. Values are expressed as mean ± SD or n (%).


^a Number of started cycles within 3 years after the start of the initial trial (TEA trial, registration no. NCT00830401) or until a live birth was obtained or treatment was stopped.

^b Student *t* test.

^c Chi-square test.

Kasius. Chronic endometritis and fertility. Fertil Steril 2011.

From Kasius et al. *Fertil Steril* 2012



Is it cost effective to do
hysteroscopy in every patient
undergoing IVF?

Hysteroscopy improves implantation regardless of abnormal findings
Endometrial injury effect

Uterine instrumentation during hysteroscopy could cause a degree of endometrial injury and provoke an immunological reaction that involves the release of cytokines and growth factors, which in turn may influence the likelihood of implantation

COST EFFECTIVE
2000 Euro per additional live birth

Hysteroscopy improves implantation only if an intracavitary lesion is corrected

NOT COST EFFECTIVE
15800 Euro per additional live birth

From Kasius et al. Hum Reprod 2011

Endometrial injury vs no injury

Table 3 Implantation rates in the intervention and control groups.

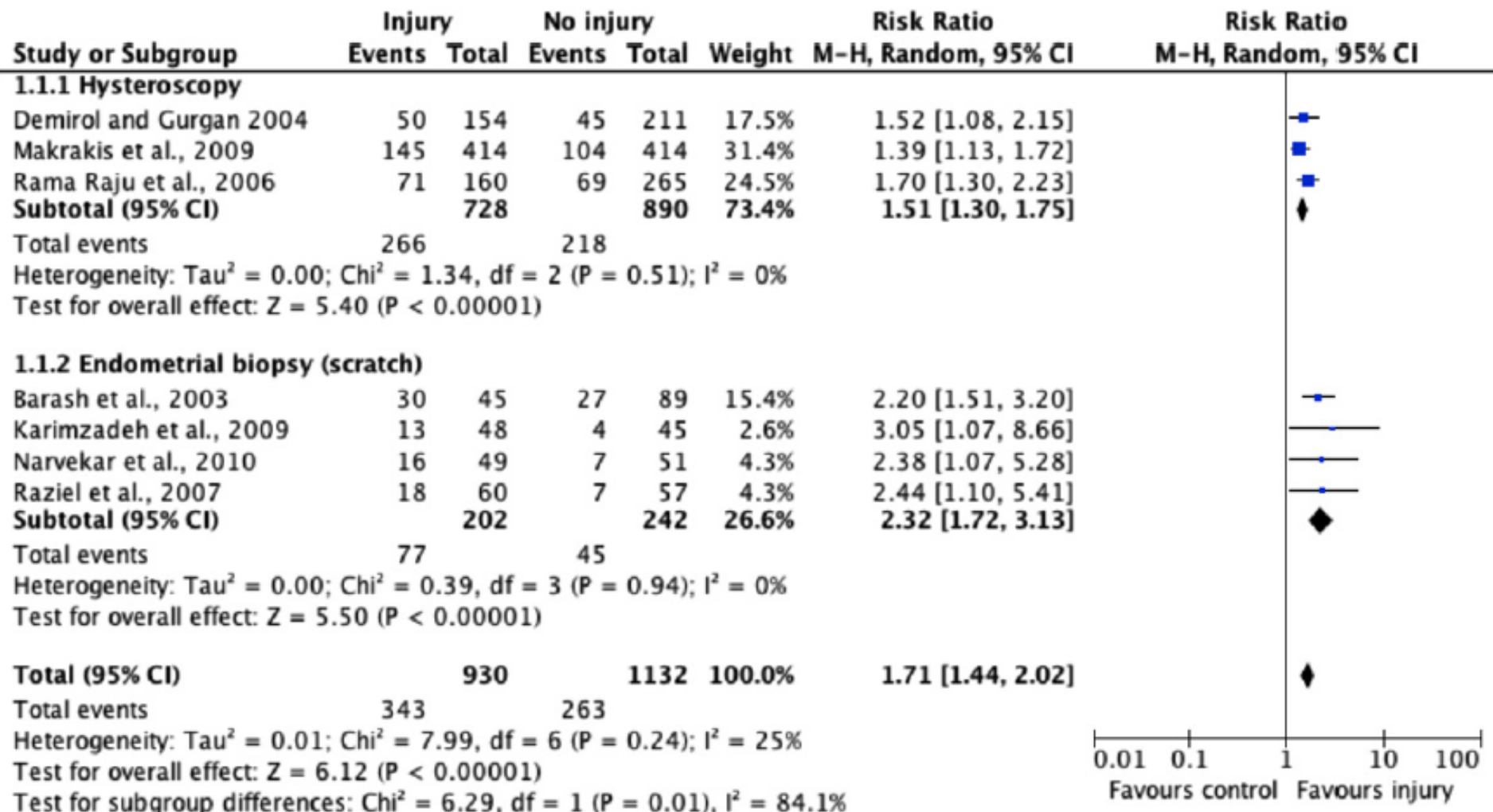
<i>Study</i>	<i>Design</i>	<i>Endometrial injury (%)</i>	<i>Control (%)</i>	<i>P-value*</i>
Barash et al. (2003)	NR	27.7	14.2	0.0001
Karimzadeh et al. (2009)	RCT	10.9	3.38	0.039
Narvekar et al. (2010)	RCT	13.07	7.1	0.04
Raziel et al. (2007)	NR	11.0	4.0	0.02


NR = non-randomized; RCT = randomized controlled trial.

*Significance level of <0.05.

From Potdar et al. RBM Online 2012

Endometrial injury and/or hysteroscopy





Can immunological disorders be
implicated in RIF?
Marketing dream academic
nightmare

Immunological disorders associated with RIF

- Autoantibodies
- Thrombophilia
- Antithyroid antibodies
- Abnormal NK cell number/function

Antibodies in IVF patients

Autoantibody	Frequency in infertile women	Infertility Association	Known associations
Antiphospholipid	Increased	Unproven	Recurrent pregnancy loss
Antithyroid	Slightly increased	Unproven	Thyroiditis, miscarriage
Antigliadin	Slightly increased	Unproven	Celiac disease
Antisperm	No difference	Unproven	Fertilization failure
Antinuclear	Slightly increased	Unproven	Autoimmune disease
Antiovarian	Slightly increased	Unproven	Ovarian failure

TABLE 1

Antiphospholipid antibodies and IVF outcome.

Outcome	Authors	Odds Ratio	(95% CI)
Pregnancy	Birdsall et al (11)	1.65	(0.50, 5.46)
	Denis et al (12)	0.91	(0.42, 1.97)
	El Roiey et al (13)	0.26	(0.04, 1.83)
	Gleicher et al (14)	1.34	(0.36, 4.95)
	Kowalik et al (15)	1.38	(0.52, 3.34)
	Kutteh et al (16)	.85	(0.21, 3.50)
	Sher et al (3)	.55	(0.13, 2.34)
Average for Pregnancy		.99	(0.64, 2.34)
Live Birth	Birdsall et al (11)	1.67	(0.50, 5.56)
	Denis et al (12)	.94	(0.44, 1.98)
	El-Roiey et al (13)	.18	(0.02, 2.14)
	Gleicher et al (14)	1.60	(0.39, 6.53)
	Kowalik et al (15)	1.10	(0.42, 2.90)
Average from Live Birth		1.07	(0.66, 1.75)

Anti-thyroid antibodies

- Is their prevalence increased in RIF vs infertile controls?

YES (22-52%)

Birkenfeld, 1994 HR
Geva, 1995 HR
Bussen, 2000 HR
Bellver, 2008 HR

- Do they reduce success rate of IVF?

Contradictory data

2 studies = yes

Geva, 1996 HR
Kim, 1998 AJRI

2 studies = no

Kutteh, 1999 HR
Negro, 2007 J Endocrinol Invest

Antithyroid AB and IVF outcome

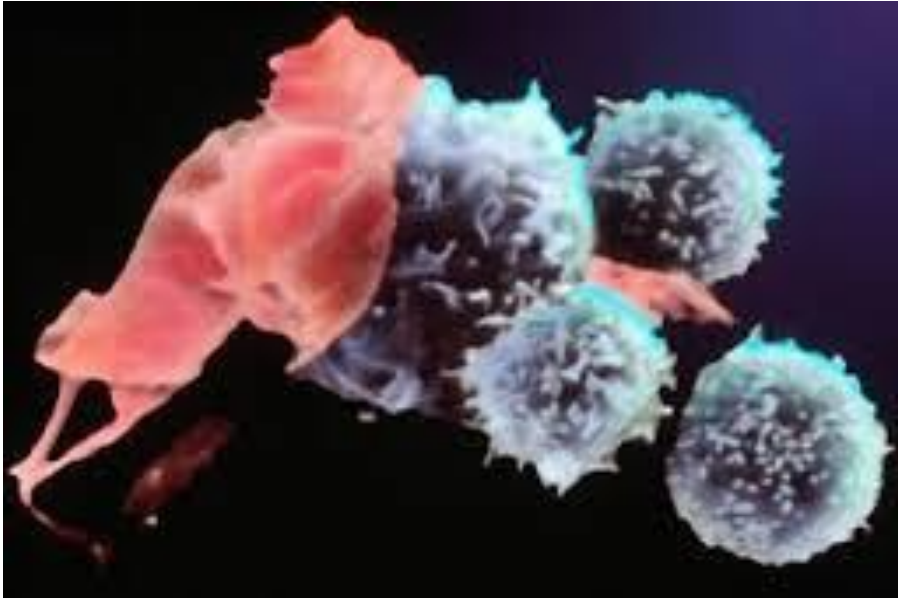
Table 2 Comparison of COS and IVF outcome between ATA+ and ATA- group

Variables	ATA+ Group	Control Group	P-value
Stimulation length(days)	11.0±1.8	10.7±1.7	0.074
Total Gn dose(IU)	2302±864	2246±736	0.885
E2 level on the day of HCG (pg/ml)	2290±1101	2342±1173	0.716
Number of re-trieved oocytes	10.9±6.1	11.8±6.9	0.166
Fertilization Rate	64.3%(729/1134)	74.6%(8848/11856)	<0.001
Number of available embryos	5.3±3.9	6.0±4.2	0.01
Number of embryo transferred	2.4±0.6	2.3±0.6	0.086
Pregnancy Rate	33.3%(52/156)	46.7%(458/981)	0.002
Implantation Rate	17.8%(66/370)	27.1%(611/2251)	<0.001
Abortion Rate	26.9%(14/52)	11.8%(54/458)	0.002

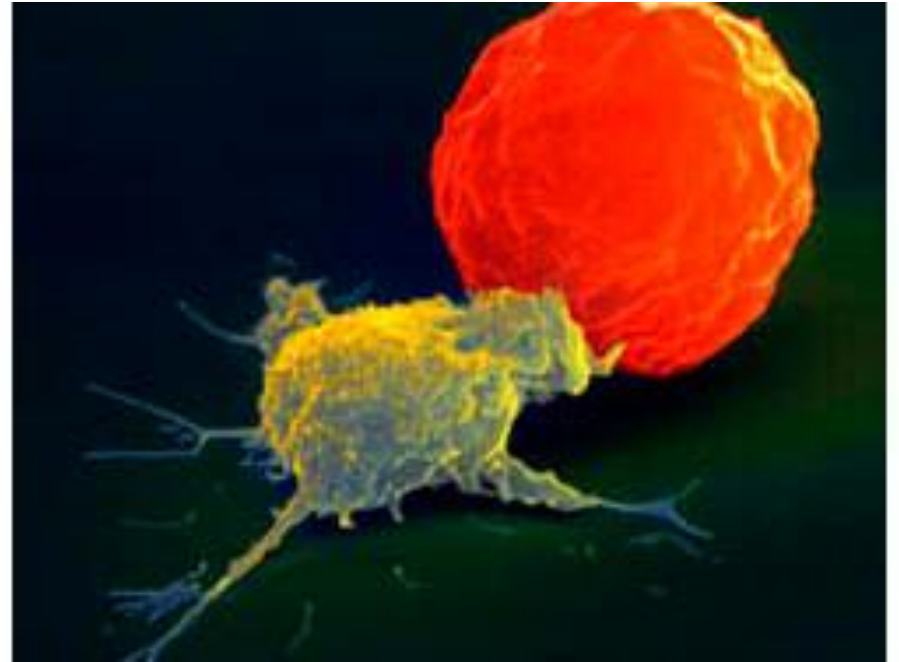
From Zhong et al. Int J Med Sci 2012

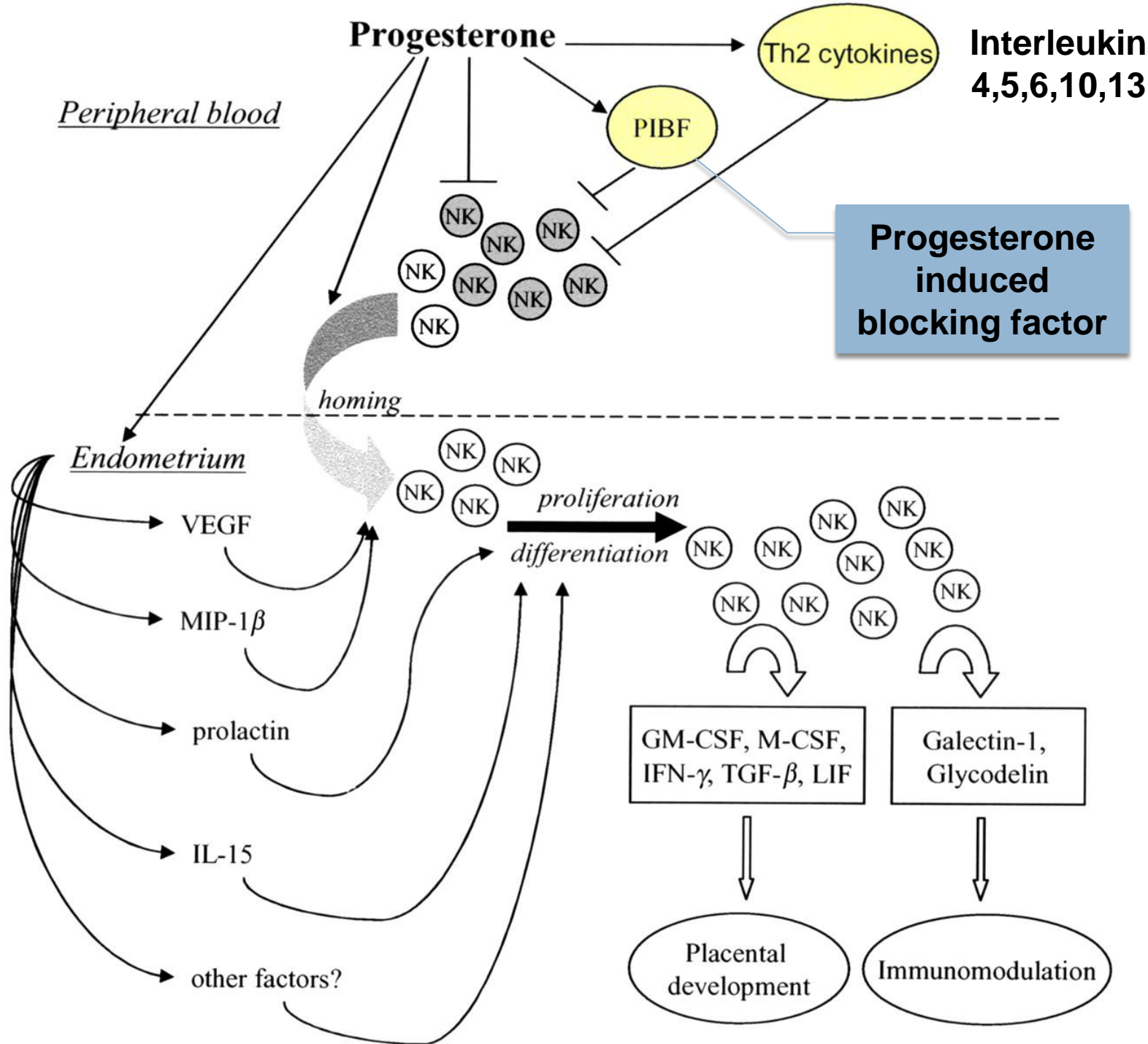
Thrombophilia

- Conflicting evidence in relation to RIF
- **Five** studies (n=600) showed higher prevalence of one or more marker in women with RIF Grandome, 2001 FS - Azem, 2004 HR - Coulam, 2006 RBM - Qublan, 2006 HR - Bellever, 2008 HR
- **One** study (n=396) showed no difference in prevalence Martinelli, 2003 Haematol



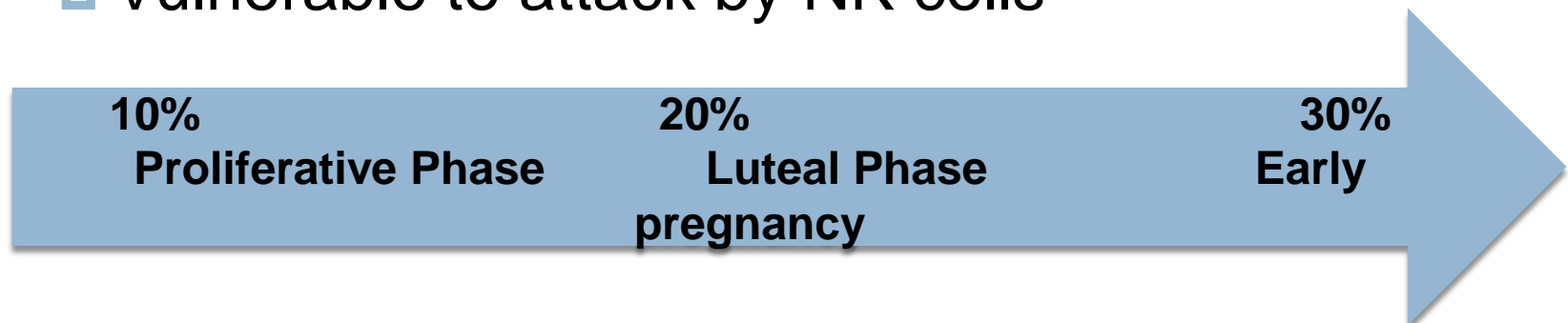
NATURAL KILLER CELLS





Natural killer cells

- NK cells do not need activation in order to kill cells missing self markers of MHC Class I antigens
- Trophoblasts do not express classical MHC I antigens
 - ▣ Immune to attack by Maternal T cells
 - ▣ Vulnerable to attack by NK cells



% of Endometrial Stromal cells that are NK cells

????

- Is it worth measuring NK cells?
- Are blood and endometrial levels concordant?
- Is there an effective treatment?
 - ▣ IVIG
 - ▣ Intralipid
- Does the treatment improve IVF success rates?



MANAGEMENT OF RIF

Management-accepted

- Remove intracavitary impediments to implantation
 - ▣ Fibroids
 - ▣ Polyps
 - ▣ Septum
- Remove hydrosalpinx
- Improve transfer technique-difficult transfers

Management-less controversial

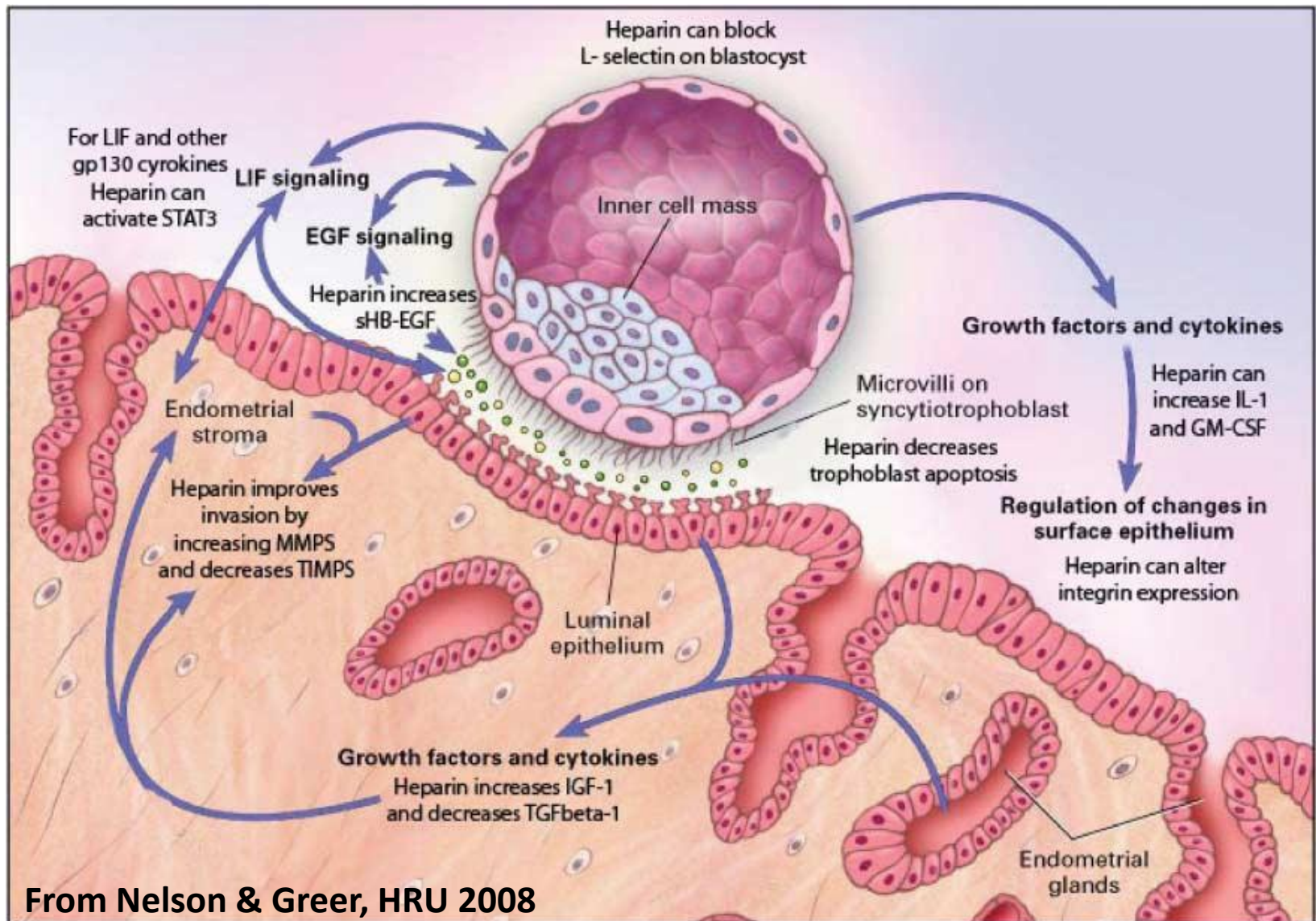
- Review stimulation protocols
- Mild stimulation
- Freeze all strategy
- Transfer at the blastocyst stage

AUTHOR	YEAR	DESIGN	PR CLEAV	PR BLAST
CRUZ	1999	RETRO	9.1	40.0
LEVITAS	2004	PRO RAND	13.7	29.4
GUERIF	2004	RETRO	19.7	27.9
BARRENETXEA	2005	RETRO	11.0	38.0

Management-more controversial

- Treatment of thrombophilia
- Treatment of thyroid autoimmunity in the euthyroid patient
- Intralipid and IVIG
- Heparin
- PGS
- Intracavitary hCG
- Intracavitary GCSF
- Multi drug approach
 - ▣ Antibiotics, aspirin, corticosteroids, multi-agent luteal phase support

Potential actions of heparin on implantation

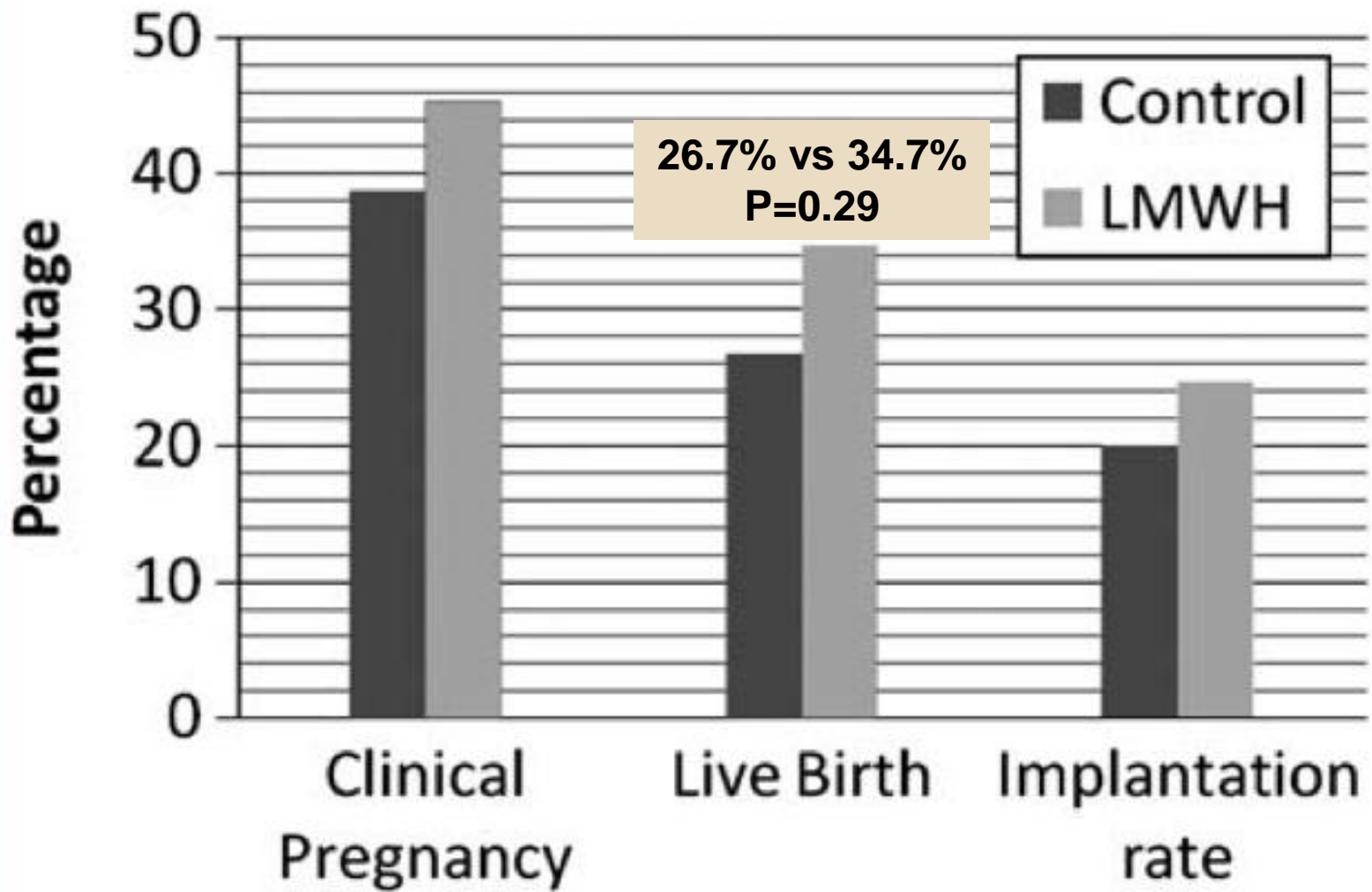


Luteal phase empirical low molecular weight heparin administration in patients with failed ICSI embryo transfer cycles: a randomized open-labeled pilot trial

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LMWH in women with RIF-with or without thrombophilia

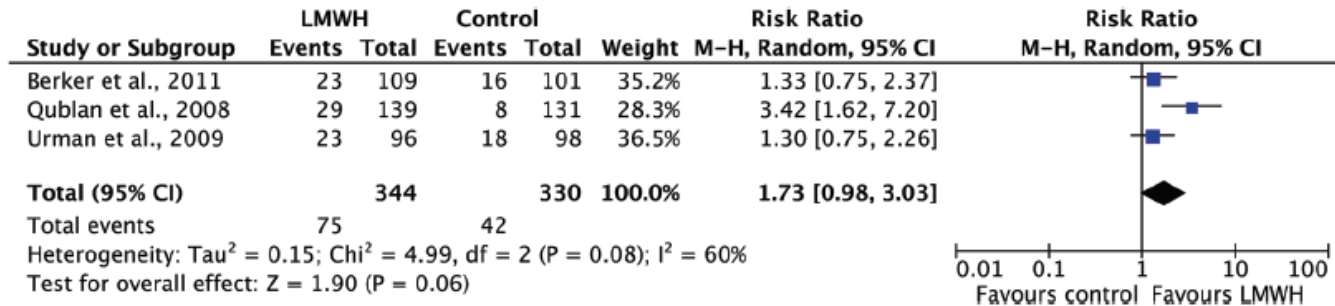


Figure 5 Implantation rate (IR) in women with ≥ 3 recurrent implantation failure and LMWH as treatment adjunct.

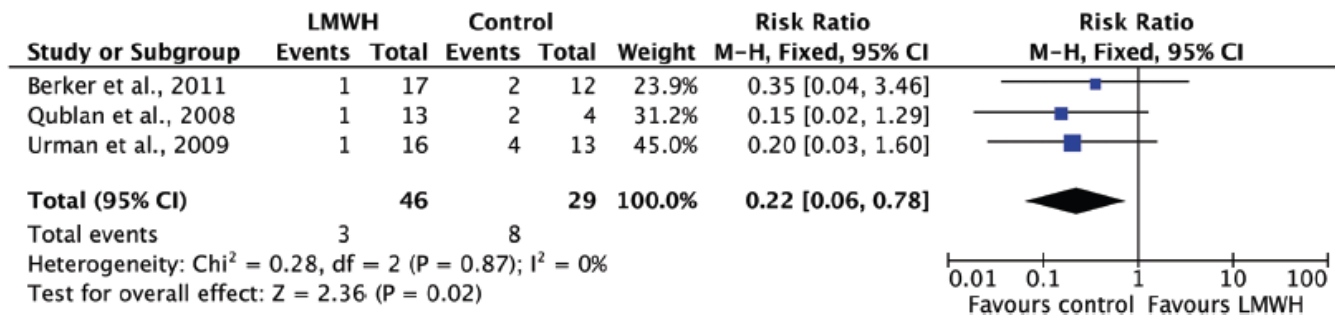
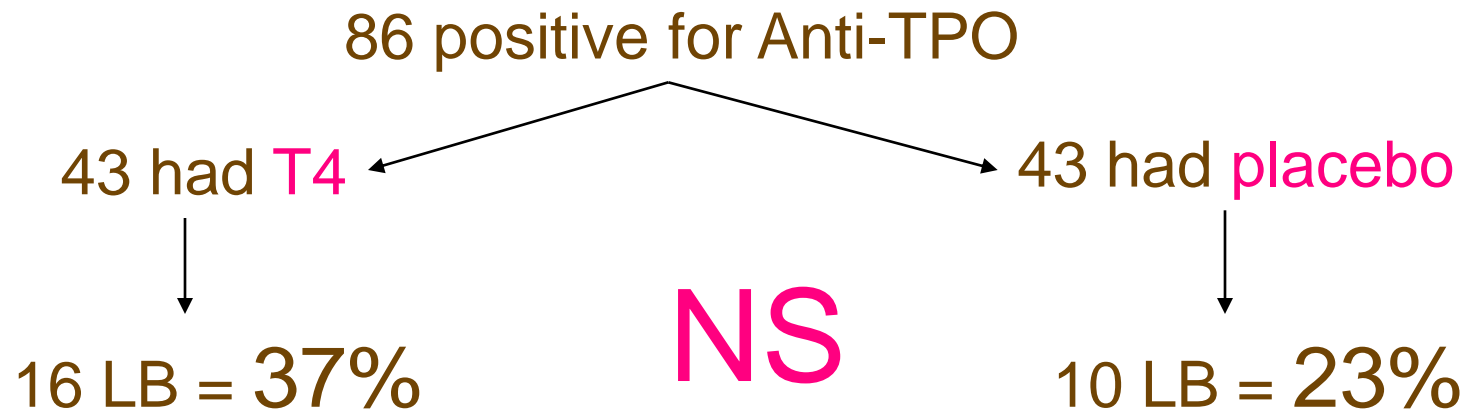


Figure 6 Miscarriage rate in women with ≥ 3 recurrent implantation failure and LMWH as treatment adjunct.

Treatment of anti-thyroid antibodies

Levothyroxine treatment in thyroid peroxidase antibody-positive women undergoing assisted reproduction technologies: a prospective study Human Reproduction Vol.20, No.6 pp. 1529–1533, 2005

Roberto Negro^{1,5}, Tiziana Mangieri², Lamberto Coppola², Giovanni Presicce², Eugenio Caroli Casavola², Riccardo Gismondi², Giancarlo Locorotondo², Paolo Caroli², Antonio Pezzarossa³, Davide Dazzi³ and Haslinda Hassan⁴



Under-powered study - 340 are required

IVIIG for treatment of RIF

- Meta-analysis of published trials showed that IVIG significantly improves the live birth rate in couples with unexplained RIF

NNT = 6

Clark et al, AJRI 2006; 23: 1-13

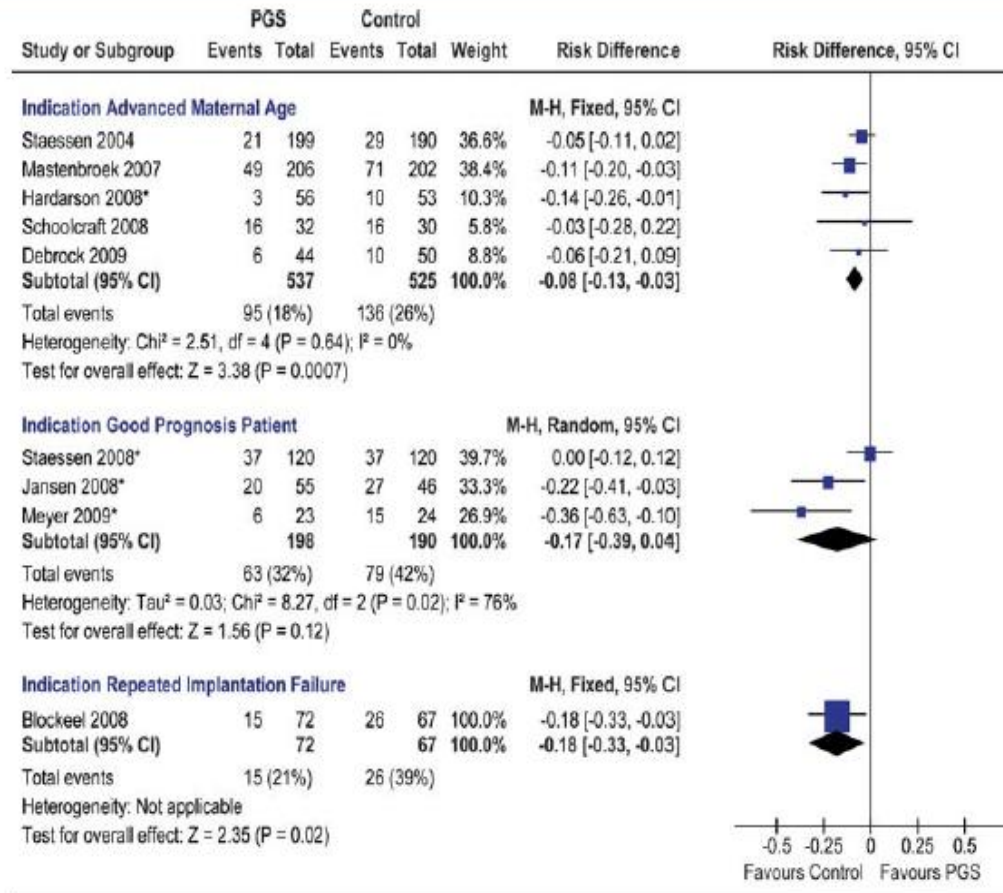
But... included 2 unpublished datasets
Few RCTs

Intralipid therapy for recurrent implantation failure: new hope or false dawn?

Shreeve and Sadek J Reprod Immunol 2012

- Intralipid
 - ▣ Contains soya oil, glycerine and egg phospholipids
 - ▣ Inhibits proinflammatory mediators specifically Th 1 cytokines
- 50% PR rate was achieved in 50 women with high order RIF undergoing Intralipid treatment (Ndukwe 2011)
 - ▣ All patients showed a reduction in their Th1/Th2 ratio
- “innovative and risk-free treatment regime”
BBC 2011

Preimplantation genetic screening



* Trial was terminated prematurely.

CI = confidence interval; M-H = Mantel-Haenszel method.

Figure 2 The effect of PGS on the live birth rate per patient.

PGS for RIF

- No beneficial effect of PGS with FISH
- No studies with newer techniques such as array CGH

Conclusions-RIF

- Only a few of the potential causes are known
- Most treatment options are experimental and empiric
- Well designed studies are urgently needed