Immunological Treatment in Reproductive Failure

Fact or Fiction?

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- INFERTILITY TREATMENTS
- REPRODUCTIVE IMMUNOLOGY
- GYNAECOLOGY CARE

The Fertility & Gynaecology Academy

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Thanks to The Organisers of The Fertility Show

Debate: To discuss or argue a matter in a formal way

Prof Lesly Regan
The president of the RCOG
Prof of O & G at Imperial College
A great patients supporter through books & TV programmes
"Reproductive Immunologist"

Prof Regan and I are both believers of Reproductive Immunology

- Prof Regan and her group were behind the evidence of treating Antiphospholipid Antibodies with Aspirin and Heparin to reduce the risk of miscarriage:

Ref:

- Unexplained Recurrent Pregnancy Loss; Saravelos & Regan, 2014
- Recurrent Miscarriage; Rai & Regan, Lancet 2006
- Impaired Expression of Endo Markers; .. Regan, .. , Mol Hum Rep 2006
- Recurrent Miscarriage: Pathology & Outcome; & Regan, C O Ob/Gyn2005
- Heparin & Aspirin Attenuate placental Apoptosis, Am J Ob/Gyn 2005
- Thrombophilia & Pregnancy Loss; J Reprod Immunology 2002
- Antiphospholipid Antibodies & Infertility; Hum Fertil 2002

Other research in RI:

- Killer-Cell Immunoglobulin-like Receptors (KIRs) and Reproductive Failure
- Hydroxychloroquine to Improve Pregnancy Outcome
- PROMISE Study: first trimester progesterone therapy in RM
- Impaired decidualisation in Recurrent Miscarriage
- Current research on G-CSF for Recurrent Miscarriage

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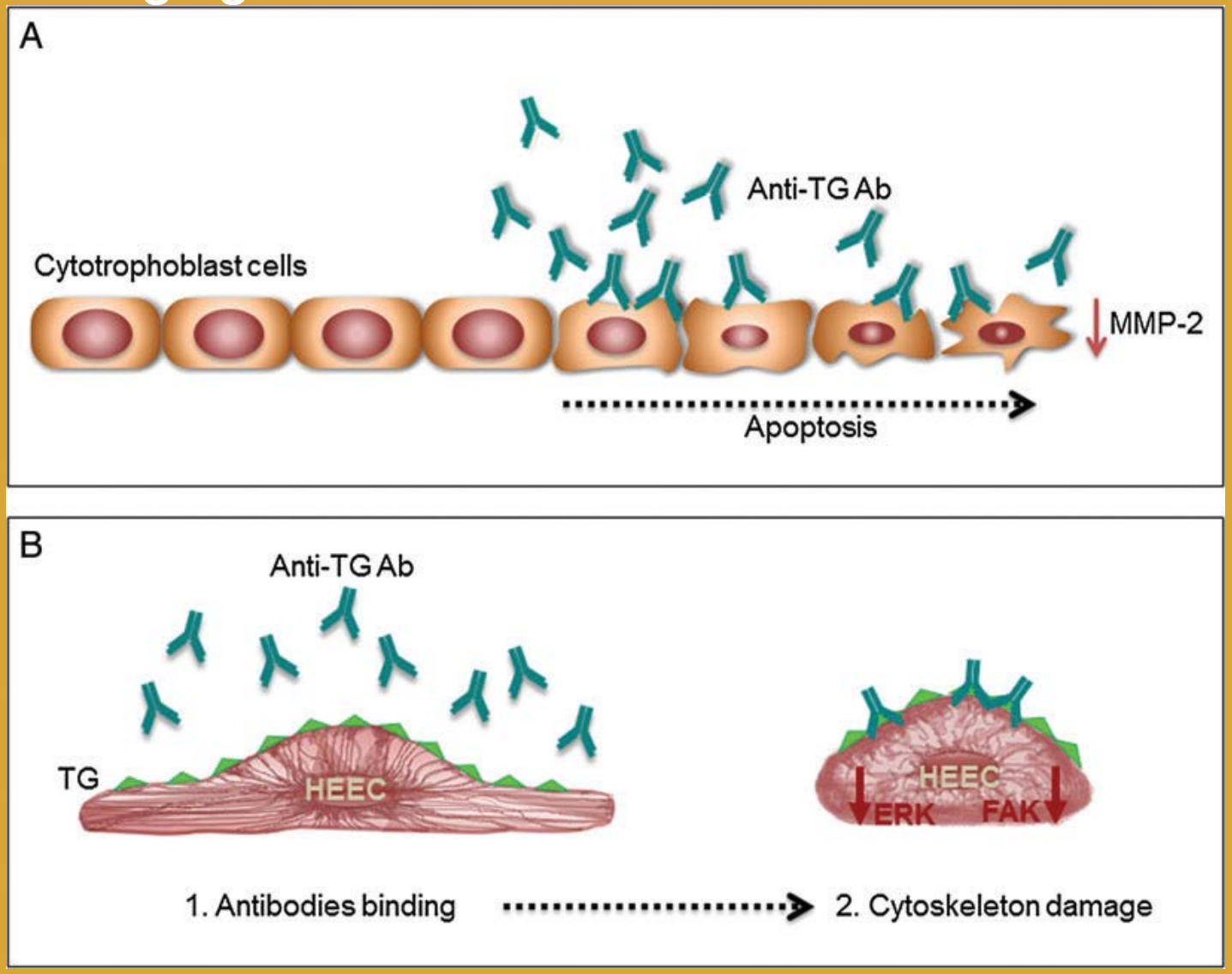
Immune Supportive Therapy: Is it the treatment for Unexplained Recurrent Miscarriage and Repeated Implantation Failure?

Does It Increase Your Chances of Having A Baby?

Damaging Effects of Antibodies

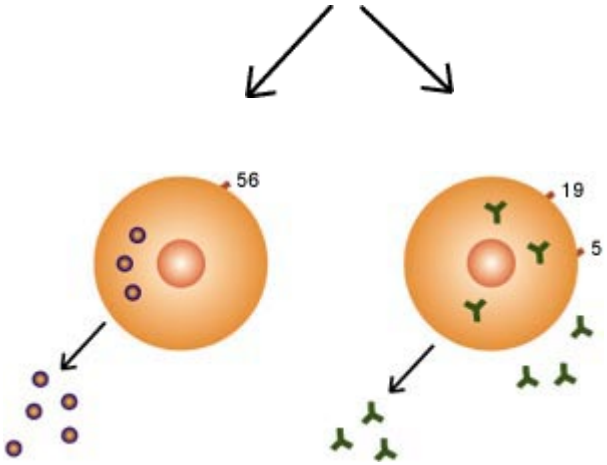


Natural Killer Cells

The Immune System
has 30 Different Kinds of Lymphocytes

↓

Two Types Can
Damage Pregnancies

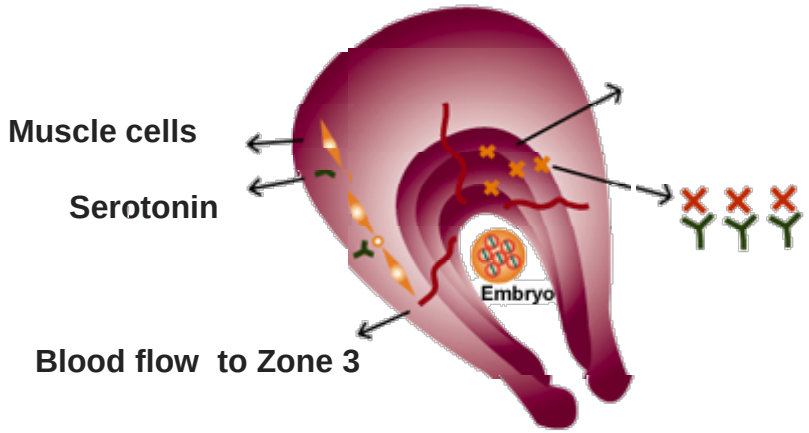


Antibodies to
Hormones

Tumour Necrosis
Factor Alpha (TNFa)

Antibodies to
Neurotransmitters

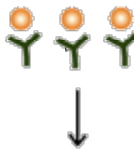
Antibodies to Neurotransmitters



Zones 1, 2 & 3 of endometrium on day of ovulation

Endorphins & enkaphlins are involved in the modeling of the 3 layers of endometrium

Antibodies to these lead to a thin "Teflon" endometrium without a blood supply



Serotonin needed to change muscle cells of uterus to accommodate a pregnancy

Antibodies to serotonin leads to a uterus that does not accommodate a pregnancy

Natural Killer (NK) Cells in Miscarriage and Implantation Failure

Increased blood NK cells in Rec M/C & Implantation Failure: CD 56 (90% dim, 10% bright)

- Aoki et al 1995
- Kwak et al 1995
- Beer et al 1996
- Fukui et at 1999
- Emmer et al 2000
- Matsubayashi et al 2001
- Coulam & Roussev (1) 2003
- Coulam & Roussev (2) 2003
- Thum et al 2004
- Skakar et al 2003
- Sacks et al 2012

Increased uterine NK cells in M/C & Implantation Failure: CD 56 (bright)

- S Quenby 1999, 2005 & 6
- Clifford & Regan 1999
- Tuckerman et al 2007, 10
- Laird et al 2005
- Nathalie Ledee 2004, 11, 16 & 17

Effect of increased NK cells and Treatment

Pathology:

- NK cells release cytokines → inflammatory reaction
- Defective angiogenesis → early onset of maternal circulation → Excessive placental oxidative stress (S Quenby)

Treatment:

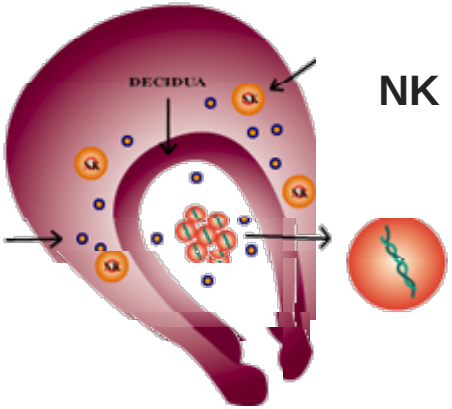
- Prednisolone reduces the u-NK cell population and activity
- Prednisolone reduces endometrial spiral artery development in RM patients.
- Prednisolone increased LB rate from 40 to 60% (S Quenby)
- Heparin and Aspirin attenuate placental apoptosis (L Regan)
- Add Pred to Aspiri & Hep: ↑LB (70.3 vs 29.7%) (Gomaa 2014)
- IVIg reduces NK cell population and activity
- G-CSF

Effect of Elevated TH1 Cytokines

- ↑ TH1 (TNFα & IFN γ) and TH17 (IL-17) in blood and decidua in patients with infertility & RM. (Piccinni 2005).
- ↑ TH1 (TNFα & IFN γ) causes trophoblast apoptosis & defective angiogenesis.
- They cause activation of complement and coagulation. (Clark 2008, Knackstedt 2001)
- ↑ PB NK cells over producing TNFα enter the implantation site blood pools. (Boomsma 2009)
- ↑ TH1/TH2 ratio is more important
- Levels can be adjusted & effect counteracted with: TNA α antagonists (Humira), IVIG, Steroids

Before Implantation

TNF Alpha

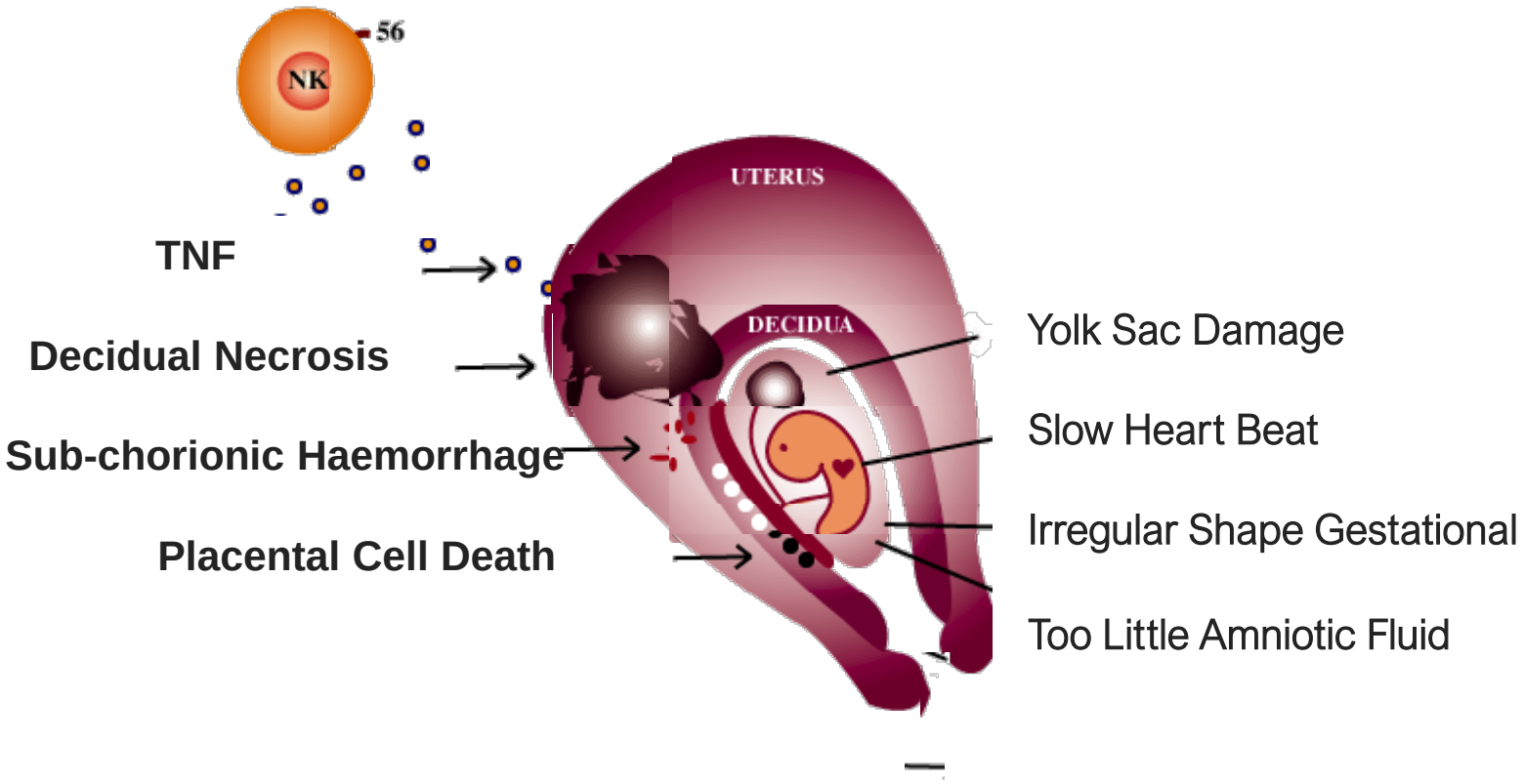


NK Cells Resident in uterus

Causes Apoptosis of the DNA in the Embryo Leading to Spot Welding of the DNA

↓

Embryo Grows Slowly and Dies.
Embryo Never Attaches.
Placental Tissue Grows With No Embryo Seen.


56
NK
TNF
Decidual Necrosis
Sub-chorionic Haemorrhage
Placental Cell Death
UTERUS
DECIDUA
Yolk Sac Damage
Slow Heart Beat
Irregular Shape Gestational
Too Little Amniotic Fluid

What is needed for a therapy to work?

- There must be an abnormal physiology
- This abnormality should be checked by a test
- The therapy must work on this pathology
- The outcome should be better after giving the therapy to the right cohort of patients
- Success should be judged by the outcome: in our case Live Birth

❑Aspirin + Heparin for Antiphospholipid Syndrome is beneficial but not for Unexplained Recurrent Miscarriage (Raj Rai & Lesley Regan, Lancet 368, 2006)

What does IVIg do?

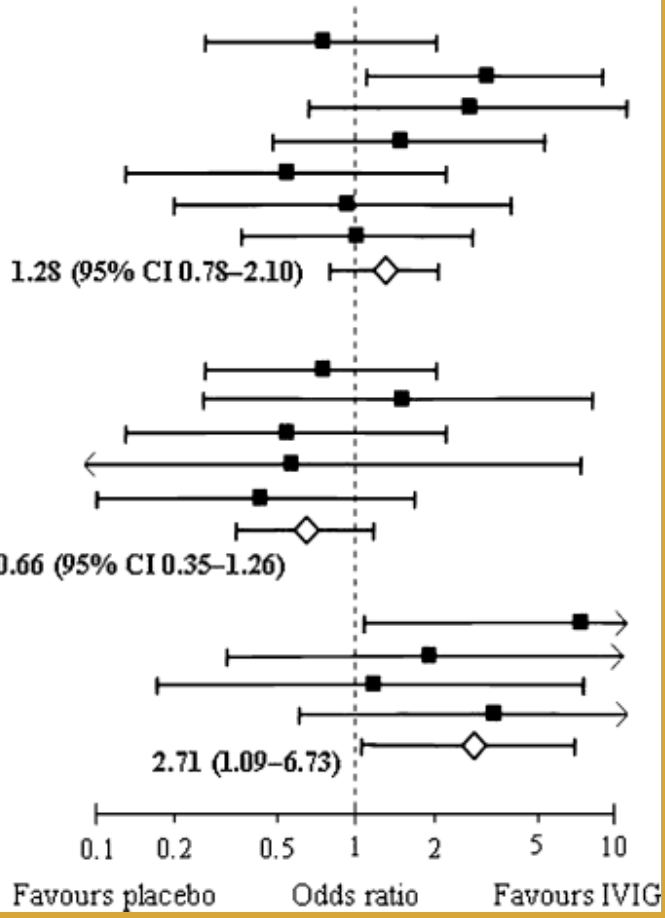
C Coulam, O Christiansen, Hutton, Clark and others

- Reduces NK cell cytotoxicity (killing activity)
- Reduces expression of pro-inflammatory T cell cytokines
- Increases the activity of T regulatory cells (Immune Tolerance)
- Suppresses B cell production of antibodies
- Actions on Fc receptors including binding of complement

Use of intravenous immunoglobulin for treatment of recurrent miscarriage: a systematic review, Hutton et al 2006

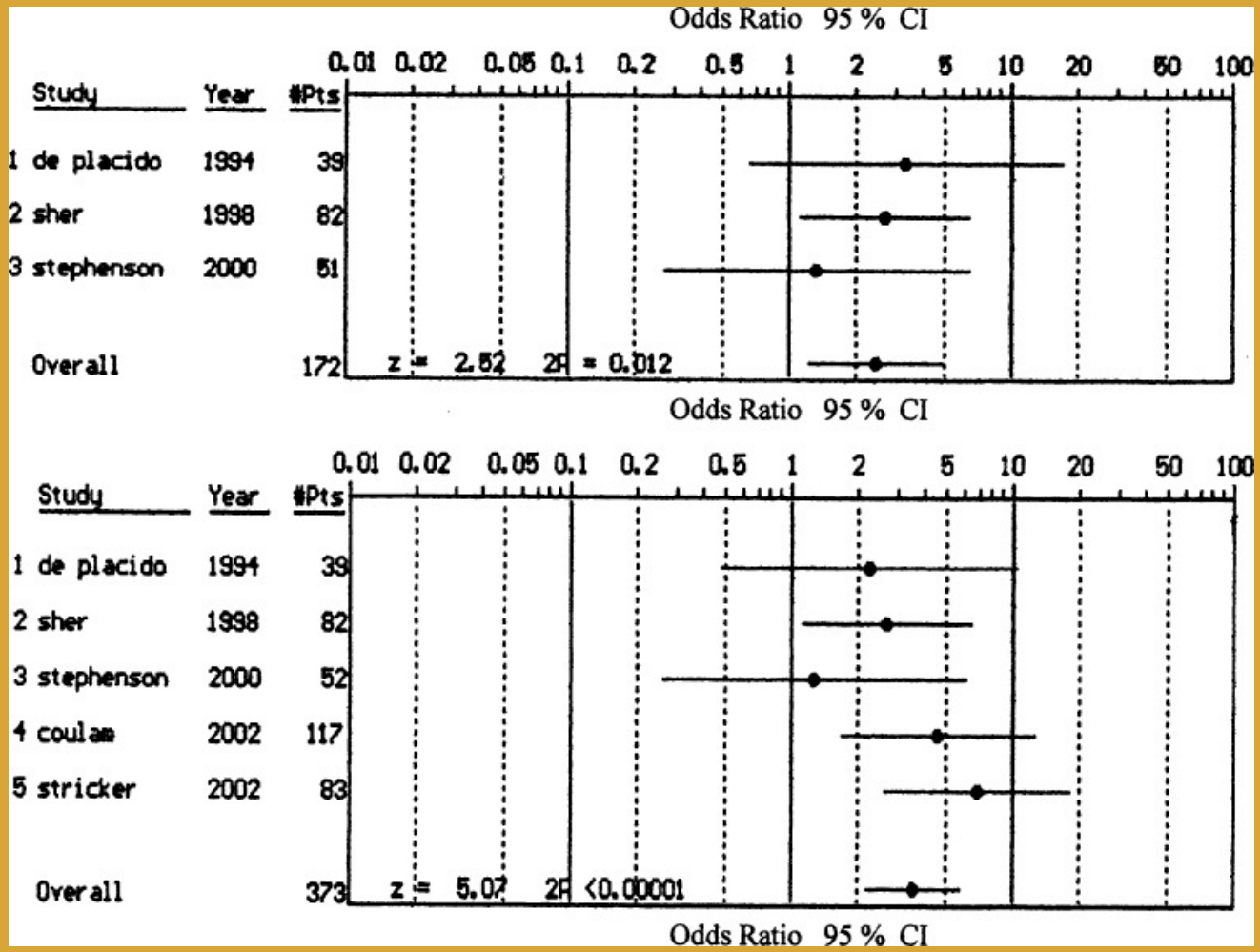
live birth rates among categories of recurrent miscarriage

	IVIG n	IVIG N	Placebo n	Placebo N
All RM				
German RSA/IVIG group[17]	20	33	21	31
Coulam *et al.*[11]	18	29	11	32
Christiansen *et al.*[10]	9	17	5	17
Stephenson[13]	12	20	10	21
Perino *et al.*[12]	16	22	20	24
Jablonowska *et al.*[14]	17	22	15	19
Christiansen *et al.*[15]	13	29	13	29
Overall	**105**	**172**	**95**	**173**
Primary RM only				
German RSA/IVIG group[17]	20	33	21	31
Stephenson[13]	5	10	4	10
Perino *et al.*[12]	16	22	20	24
Jablonowska *et al.*[14]	9	11	8	9
Christiansen *et al.*[15]	7	17	10	16
Overall	**57**	**93**	**63**	**90**
Secondary RM only				
Christiansen *et al.*[10]	9	14	2	10
Stephenson[13]	7	10	6	11
Jablonowska *et al.*[14]	8	11	7	10
Christiansen *et al.*[15]	6	12	3	13
Overall	**30**	**47**	**17**	**44**



Is intravenous immunoglobulin (IVIG) efficacious in early pregnancy failure? A critical review and meta-analysis for patients who fail in vitro fertilization and embryo transfer (IVF)

David A. Clark, Carolyn B. Coulam, and Raphael B. Stricker; 2006



Double-blind randomized controlled trial (RCT) of intravenous immunoglobulin (IVIG) in idiopathic secondary recurrent miscarriage and conclude 'no benefit was found'. Stephenson et al. (2010)

Methodological Flaws:

- The planned sample size was not achieved. No Conclusion
- Heterogeneous group of patients. Idiopathic
- Using Gamimune: Less potent (Clark et al 2006)
- Exclusion of patients with immune issues
- Exclusion from the meta-analysis two trials by Christiansen et al (1995 & 2002) because they included patients with immune abnormalities!!!
- Adding these two trials will make the meta analysis significant in favour of IVIg.

Is intravenous immunoglobulin (IVIG) efficacious in early pregnancy failure? A critical review and meta-analysis for patients who fail in vitro fertilization and embryo transfer (IVF)

David A. Clark,, Carolyn B. Coulam, Raphael B. Stricker (2006)

Table IV.

Classification of Outcome of Controlled Trials of IVIG in Reproductive Failure

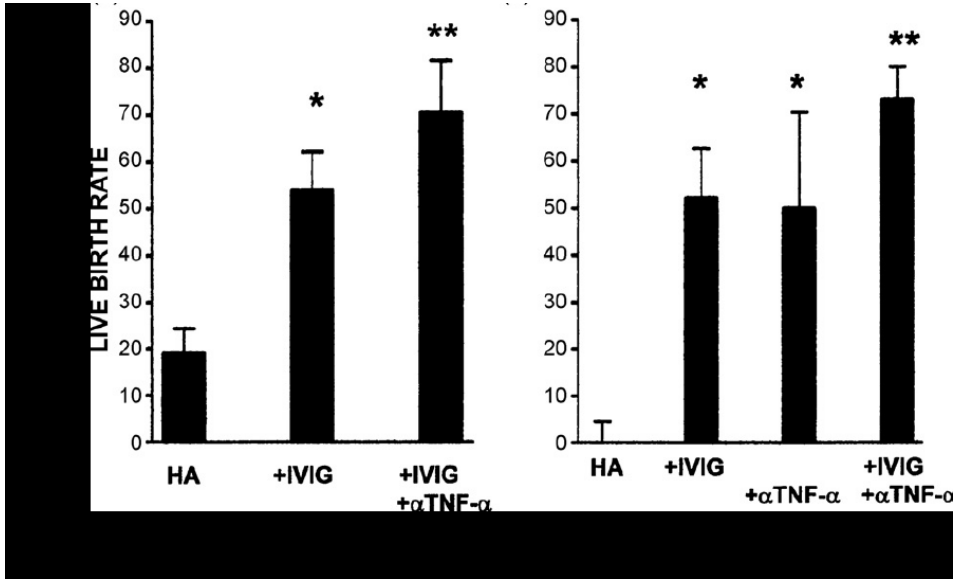
	Pre-ovulatory IVIG start		IVIG started post-pregnancy	
Outcome	**RSA trials**	**IVF failure trials**	**RSA trials**	**IVF failure trials**
Positive[a]	Coulam [95] (48)	Sher [82] (10)	None	None
	Kiprov [35] (49)	de Placido [39] (9)		
	Stricker [47] (50)	Stricker [61] Table III		
		Coulam [107] Table II		
Negative	Stephenson [39] (51)	Stephenson [51] (4)	German Grp [64] (52)	None
			Christiansen [34] (53)	
			Christiansen [58] (54)	
			Perino [46] (55)	
			Jablonowska [41] (56)	

Debate: Should Immunotherapy be Used? Intravenous Immunoglobulin: Yes Carolyn Coulam 2014

Trial	n	IVIg start	Selection	LB (p< 0.05)
Moraru et al 2012	157	Before pre	Immune test	Yes
Coulam et al 1995	95	Before pre	Ob history	Yes
Kiprov et al 1996	35	Before pre	Immune test	Yes
Stricker et al 2000	47	Before pre	Immune test	Yes
Stevenson et al 98	39	Before pre	Ob History	No
Mueller-Eckhart 94	64	After pre	Ob History	No
Christiansen 2002	34	After pre	Ob History	No
Christiansen 1995	58	After pre	Ob History	No
Perino et al 1997	46	After pre	Ob History	No
Jablonowska 1999	41	After pre	Ob History	No

Treatment with tumour necrosis factor inhibitors and intravenous immunoglobulin improves live birth rates in women with recurrent spontaneous abortion (Winger & Reed 2008) and in Infertile IVF Patients (Winger et al 2009)

- IVIg significantly improved LB rate in patients with high NK cell & TH1/TH2 cytokine ratio.
- Adding Humira to IVIg improved the LB rate further.
- Patients with border line elevated TH1/TH2 cytokine ratio were given IVIg only
- Patients with remarkably high TH1/TH2 cytokine ratio were given Humira in addition to the IVIg



The Role of NK Cells in Human Fertility

RCOG Scientific Impact Paper No. 53 Dec 2016

- PB NK cells may migrate into the endometrium
- Biological activity of PB NK & uNK cells is more significant
- Studies suggest that PB NK & uNK may play a role in RM
- TH2 cytokines favour successful pregnancy, while TH1 cytokines are considered detrimental
- No indication to test for NK cells in infertility BUT uNK cell testing in RM & RIF is debatable pending further evidence
- Preliminary data suggests a positive effect of prednisolone

Immune Tolerance Prevents Immune Rejection of the Foetus Sarah Robertson group 2009

- T-reg cells increase in early pregnancy and decline prior to labour
- They even increase prior to pregnancy following intercourse
- Miscarriage is associated with low blood and endometrial levels
- Recurrent miscarriage is associated with low blood levels
- Unexplained infertility is associated with low endometrial levels

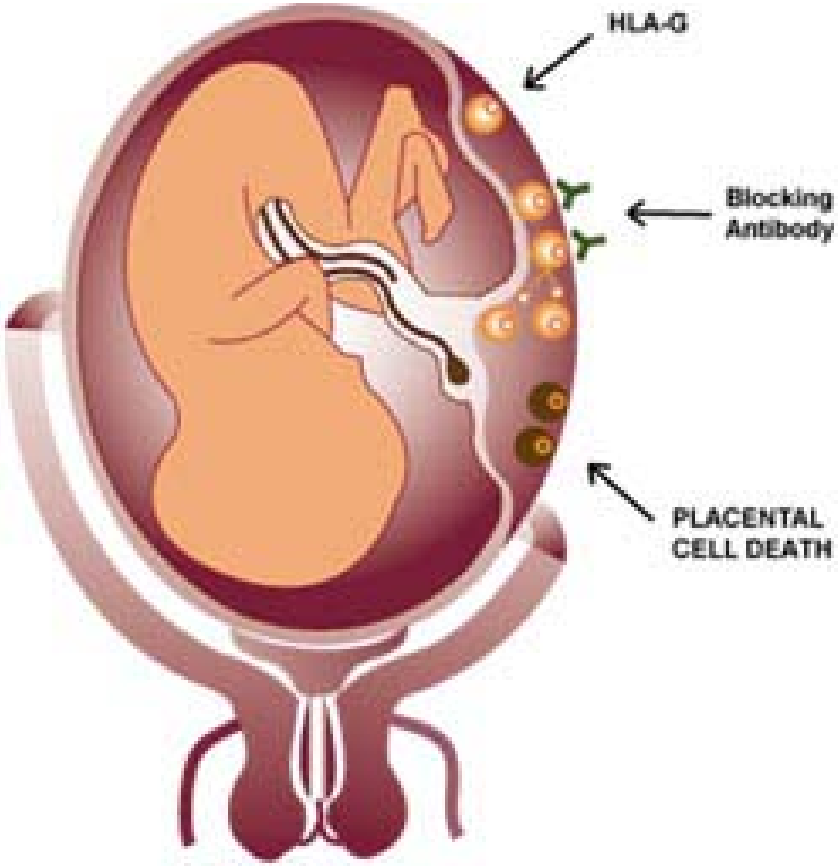
Possible therapy to induce T-reg cells:

Lymphocyte Immune Therapy

G-CSF

Paternal Leukocyte Antibodies

- **HLA-G:** Message sent from father to stimulate blocking antibody.
- **Blocking Antibody:** Protects and stimulates the growth of placental cells.
- **Placental Cell Death:** Consequences of low blocking antibody.



Lymphocyte Immune Therapy

- First used to induce immune tolerance in kidney transplant
- RCT by Mowbray published 1985
- Ober's trial 1999: -ve outcome
- Media attention, FDA allowed LIT only in Research Setup
- Pandy & Agrawal 2004: extremely +ve effect
- Meta-analysis: +ve outcome even including Ober trial
- LIT success is related to the dose of CD 200 marker in LIT
- CD 200 is significantly reduced with storing the sample at 4º overnight (Ober) and increase at 37º C (Pandy & Agrawal)

Use of G-CSF for Treatment of Unexplained Recurrent Miscarriage: a Randomized Controlled Trial. (Scrapellini & Sbracia 2009)

- G-CSF group: 82% LB Vs 48.5% in Placebo group Significant Difference: p = 0.006, OR 5.1 (95% CI 1.5-18.4)
- Higher levels of β-hCG: a +ve effect on trophoblast growth and placental metabolism (McCraken et al 1996 & 99)
- G-CSF induces TH2 response (Pan et al 2011)
- G-CSF inhibits NK cells (Schlahsa et al 2011)
- G-CSF induces immune tolerance
- G-CSF for patients with RIF increased pregnancy rate to 73.8% in D5 & 42% in D2 ET. (Wolfgang Wurfel 2010)

The Uterine Immune Profile may help women with Repeated Unexplained Embryo Implantation Failure after IVF. Nathalie Ledee 2015

Over Activation 56.6%	Normal Balance 18.3%	Low Activation 25%
1- Prednisolone &/or intralipid 2- High dose Vit E 3- High dose of P4 ± E2 Luteal support	Probably non immune causes	1- Endometrial scratch the month before IVF. 2-hCG supplement after egg collection 3- Sexual Intercourse after ET
LB: 36.8%	**LB: 19.4%**	**LB: 46.5%**

Complement Activation, a Threat to Pregnancy (2017)

Can Statins Prevent Pregnancy Complications (2014)

Pravastatin Improves Pregnancy Outcome in Obstetric Antiphospholipid Syndrome Refractory to Antithrombotic Therapy (2016)

Prof. Girardi

Complement Inhibition by Hydroxychloroquine Prevents Placental and Foetal brain abnormalities in Antiphospholipid Syndrome (2016)

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Immune Supportive Therapy: Is it the treatment for patients with Recurrent Miscarriage or Repeated Implantation Failure due to Immune System Imbalance?

Does It Increase Your Chances of Having A Healthy Baby?

YES

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Prof Sir Magdi Yacoub once said

I am grateful for those who agreed with my ideas as they encouraged me to work more

But I am most grateful for those who disagreed with my ideas as they made me work harder to find the truth

Thank you