



OVARIAN CLUB VIII

BUILDING A BRIDGE BETWEEN SCIENCE
AND CLINICAL PRACTICE

4-6 NOVEMBER, 2016 • PARIS, FRANCE



The Women's Clinic

婦產科中心

Dr. Milton Leong
The IVF Clinic Hong Kong

A structured immuno-suppression treatment for repeated implantation failure?

Disclosure information: Nothing to declare

<http://oc2016.cme-congresses.com/>



The Women's Clinic

婦產科中心

A structured immuno-suppression treatment for repeated implantation failure?

Milton Ka Hong Leong MDCM DSc(McGill)

FRCS(C) FRCOG FACOG FHKCOG

Specialist in Reproductive Medicine

Adjunct Professor, Department of O & G, McGill University, CANADA

Medical Director, The Women's Clinic and The IVF Clinic Hong Kong

Co-Founder, www.ivf-worldwide.com



The Women's Clinic

婦產科中心

- Takes average 25 eggs to make a baby
- 50% of pregnancies are lost before missing a menstrual period
- Pregnancy loss due to chromosomal abnormalities and implantation failure
- Implantation is endometrium and related cellular and molecular changes encouraging tolerance
- Implantation failure when embryos and maternal environment do not fit

IMPLANTATION



The Women's Clinic

婦產科中心

- A complex process where the interplay of various factors resulting in the host (endometrium) to accept a semi-allogenic embryo to develop and grow within it. These factors include cellular changes, hormones, vascular adaptation, as well as the interplay of cytokines, growth factors, mRNA, as well as gamete and embryo quality.
- 75% conception loss is due to failure of implantation

WILCOX NEJM 1988



The Women's Clinic

婦產科中心

Implantation depends on the synchronization of factors:

- quality of embryos
 - optimal culture conditions
 - receptivity of the endometrium
 - maternal immune system
-
- It is of utmost importance to understand how the initial inflammatory response during the implantation period is controlled to protect the semi-allogenic fetus

Basis of Immuno-modulatory Treatment

Description of inflammation vs implantation

Beer, Scott, Coulam, Kwak and many others

APCA and lymphocyte transfusion

Mulbry, Reagan et al 1980's

Chicago "Team" (Beer and others set up an "immunology panel" – LIT USA

Basic science studies and clinical trials IVIG, intralipid, steroids led to a structured immuno-modulatory treatment for RPL and RIF



The Women's Clinic

婦產科中心



The Women's Clinic

婦產科中心

Immunological blood tests

1. Anti-phospholipid antibodies (APA)
2. Natural killer(NK) cells
3. Th1 and Th2 cytokines
4. Anti-nuclear Antibodies (ANA)
5. Plasminogen Activator Inhibitor (PAI)
6. Antithyroid antibodies
7. Lupus-like anticoagulant

Immunological tests

8. HLA-G
9. Sperm DNA integrity assay
10. Thrombophilia panel
 - factors II, V, VIII
 - b-fibrinogen
 - methylenetetrahydrofolate reductase (MTHFR)
11. Y-Chromosome deletion assay
12. Vitamin D



The Women's Clinic

婦產科中心

History and time line

1993 – 1995 collaboration with St Mary's Hospital London and Professor Mulbry started program of APCA testing and Lymphocyte transfusion treatment

55 patients with repeated pregnancy loss from 3-12x

Negative tested for APCA – 48

Treated with LIT a total of 62 cycles, natural, IUI, and IVF

10 pregnancies : 7 miscarriages, 1x34 weeks premature, 2 term deliveries

Stopped Treatment 1995

LIT banned in USA 2003



The Women's Clinic

婦產科中心

History and time-line

- No treatment from 1995-2005, tests not available!
- Circa 2005 started collaborating with Dr Bernard Chan, hematologist treating repeated miscarriages and then expended to repeated IVF failures
- Circa 2008 more focus on IVF failures, and worked out sending blood tests to Rosalind Franklin Medical School, Chicago
- 2010 Personal treatment based on “Chicago School”
- 2013 Started replacing IVIG with intralipid



The Women's Clinic

婦產科中心

Bridging science and practice



The Women's Clinic

婦產科中心

Early cases – Proof of Concept



The Women's Clinic

婦產科中心

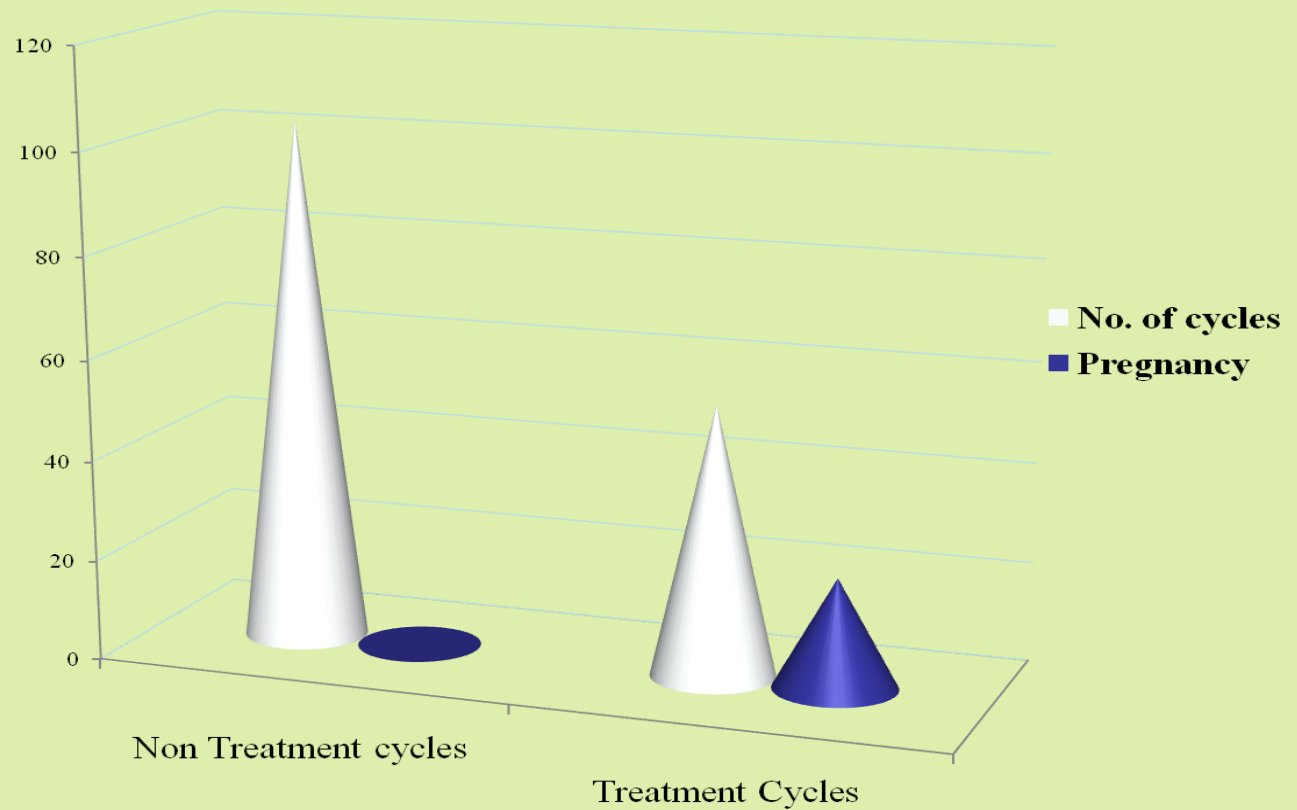
Early cases – Brief Clinical Summary

- 40 patients treated in 2010-11
- Age ranging from 30-44
- History of repeated implantation failures
(2 patients with repeated failure in IUI)
- Immunological blood tests were performed
- Treatment with Prednisolone, IVIG, Clexane and Aspirin were prescribed



The Women's Clinic

婦產科中心



Reference cycles (104) no pregnancy;
Treatment cycles 54, 22 pregnant; 1 miscarried
Pregnancy rate 41% % miscarriage = 4.5%



The Women's Clinic

婦產科中心

Immunology compromised patients

- Series II 2011-2013
- All patients referred for repeated IVF or very early pregnancy failures (EPF)
- EPF = biochemical, ectopic, empty sac pregnancies
- Age 35-47
- All have at least 3 failures, and more than 8 embryos replaced
- All underwent the same group of immunological tests done by the Rosalind Franklin reproductive centre laboratory



The Women's Clinic

婦產科中心

Immunology compromised patients

- All had at least one failed IVF treatment in our own center before entering trial
- Acting as their own control, all had IVF treatment with the same protocol before without and with immuno-suppression treatments
- A no selection prospective study, only entry point was positive (abnormal) immunologic test panel



The Women's Clinic

婦產科中心

Immunological blood tests taken

1. Anti-phospholipid antibodies (APA)
2. Natural killer(NK) cells
3. Th1 and Th2 cytokines
4. Anti-nuclear Antibodies (ANA)
5. Plasminogen Activator Inhibitor (PAI)
6. Antithyroid antibodies
7. Lupus-like anticoagulant
8. Recently added Vitamin D

Immunology tests Reports



The Women's Clinic

婦產科中心

Clinical Immunology Lab
 (847) 578-3444 E-mail: clinlab@rosalindfranklin.edu
 3333 Green Bay Road
 NORTH CHICAGO, IL 60064

Kenneth Be

| | RESULT | UNITS |
|---------------------|----------|--------------|
| | IN RANGE | OUT OF RANGE |
| Polymorphism | | |
| etection | NORMAL | |

tor inhibitor-1 (PAI-1) is an essential regulatory component of fibrinolytic pathway
 eletion gene polymorphism at 675 bp is related to levels of PAI-1 protein. Homozy
) is associated with higher levels of PAI-1 protein and increased risk for thrombosi
 ype; HETEROZYGOUS MUTATED represents a 4G/5G genotype; HOMOZYGO
 e.

e characteristics of this test were determined and are monitored by the Clinical Im
 re RFUMS/The Chicago Medical School. The use of this test has not been cleared
 A for diagnostic testing.

Clinical Immunology Lab
 (847) 578-3444 E-mail: clinlab@rosalindfranklin.edu
 3333 Green Bay Road
 NORTH CHICAGO, IL 60064

CLIA ID# 14D06
Kenneth Beaman, Ph.D., D(A)

| TEST NAME | RESULT | UNITS | REFERENCE RANGE |
|---|----------|--------------|-----------------|
| | IN RANGE | OUT OF RANGE | |
| Anti-phospholipid Antibody Panel | | | |
| IgM-Cardiolipin | NEGATIVE | | NEGATIVE |
| IgM-Phosphoethanolamine | POSITIVE | | NEGATIVE |
| IgM-Phosphoinositol | NEGATIVE | | NEGATIVE |
| IgM-Phosphatidic Acid | NEGATIVE | | NEGATIVE |
| IgM-Phosphoglycerol | NEGATIVE | | NEGATIVE |
| IgM-Phosphoserine | NEGATIVE | | NEGATIVE |
| IgG-Cardiolipin | NEGATIVE | | NEGATIVE |
| IgG-Phosphoethanolamine | NEGATIVE | | NEGATIVE |
| IgG-Phosphoinositol | NEGATIVE | | NEGATIVE |
| IgG-Phosphatidic Acid | NEGATIVE | | NEGATIVE |
| IgG-Phosphoglycerol | NEGATIVE | | NEGATIVE |
| IgG-Phosphoserine | NEGATIVE | | NEGATIVE |
| IgA-Cardiolipin | NEGATIVE | | NEGATIVE |
| IgA-Phosphoethanolamine | NEGATIVE | | NEGATIVE |
| IgA-Phosphoinositol | NEGATIVE | | NEGATIVE |
| IgA-Phosphatidic Acid | NEGATIVE | | NEGATIVE |
| IgA-Phosphoglycerol | NEGATIVE | | NEGATIVE |
| IgA-Phosphoserine | NEGATIVE | | NEGATIVE |

Notes: BORDERLINE has an approximate titer of 1:50 and should be considered as an ANA of 1:40, that is suspicious but not clearly positive.
 POSITIVE results have titers equal to 1:100 to 1:200.
 HIGH POSITIVE results have an equivalent titer of 1:400 or greater and like titers of 1:320 or 1:640 in the ANA test are indicative of a frank disease process.


This test was developed by the Clinical Immunology Laboratory at the RFUMS/The Chicago Medical School. The performance characteristics of this test were determined and are monitored by the Clinical Immunology Laboratory. However, The use of this test has not been cleared or approved by the U.S. FDA.

Immunology tests Reports



The Women's Clinic

婦產科中心



Clinical Immunology Lab
 (847) 578-3444 E-mail: clinlab@rosalindfranklin.edu
 3333 Green Bay Road
 NORTH CHICAGO, IL 60064

CLIA ID# 14D00
 Kenneth Beaman, Ph.D., D(A)

| NAME | RESULT | | UNITS | REFERENCE RANGE |
|-------------------------|----------|--------------|-------|-----------------|
| | IN RANGE | OUT OF RANGE | | |
| Assay Full Panel | | | | |
| | 27.1 | | % | 10.0-40.0 |
| | 23.4 | | % | 5.0-30.0 |
| | | 21.3 (H) | % | 3.0-20.0 |
| 12.5 mg/ml, 50:1** | 28.0 | | % | |
| 12.5 mg/ml, 25:1** | 29.1 | | % | |
| 6.25 mg/ml, 50:1** | 23.1 | | % | |
| 6.25 mg/ml, 25:1** | 19.4 | | % | |
| 3 | 62.0 | | % | 60.0-85.0 |
| 19 | | 25.9 (H) | % | 2.0-12.0 |
| 56 | 9.2 | | % | 2.0-12.0 |
| 19+cells_CD5+ | | 21.7 (H) | % | 5.0-10.0 |

** > 10% reduction in killing at each effector/target ratio.

This test was developed by the Clinical Immunology Laboratory at the RFUMS/The Chicago Medical School. The performance characteristics of this test were determined and are monitored by the Clinical Immunology Laboratory. The use of this test has not been cleared or approved by the U.S. FDA.

TH2 Intracellular Cytokine Ratios

| | | | |
|--------------------|----------|-------|-----------|
| i:IL-10 (CD3+CD4+) | 45.5 (H) | ratio | 13.2-30.6 |
| i:IL-10 (CD3+CD4+) | 22.1 (H) | ratio | 5.8-20.5 |

This test was developed by the Clinical Immunology Laboratory at the RFUMS/The Chicago Medical School. The performance characteristics of this test were determined and are monitored by the Clinical Immunology Laboratory. The use of this test has not been cleared or approved by the U.S. FDA.



PATHLAB
 MEDICAL LABORATORIES LTD
 醫學化驗所

Antibodies (by IFA) 抗核因子

Reference range:
 Healthy adults (95 percentile) Titre < 100

Note: Reference notes updated Sept 5, 2011

Reference notes:
 Guideline to ANA prevalence in autoimmune disease:

| | |
|--|-----------|
| Systemic lupus erythematosus: | |
| Active | 95 - 100% |
| Inactive | 80 - 100% |
| Drug induced lupus erythematosus | 100% |
| Mixed connective tissue disease (MCTD, Sharp Syndrome) | 100% |
| Rheumatoid arthritis | 20 - 40% |
| Other rheumatic diseases | 20 - 50% |
| Progressive systemic sclerosis | 85 - 95% |
| Polymyositis and dermatomyositis | 30 - 50% |
| Sjogren's syndrome | 70 - 80% |
| Chronic active hepatitis | 30 - 40% |
| Ulcerative colitis | 26% |

The diagnostic value of autoantibodies against cell nuclei (ANA) is proven for many autoimmune diseases, but not exclusively those of the rheumatic forms. Interference includes patients having high antibody levels that may mask the pattern and titer of ANA. Positive ANA testing may be more meaningful interpreted with additional tests, such as Anti ds-DNA (SLE) and ENA antibodies for the differentiation of diseases.

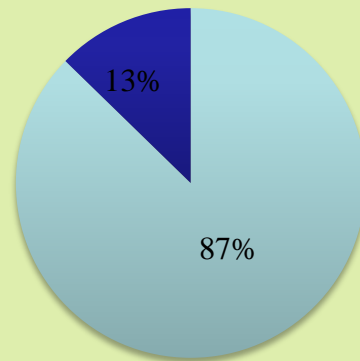
Immunological tests results



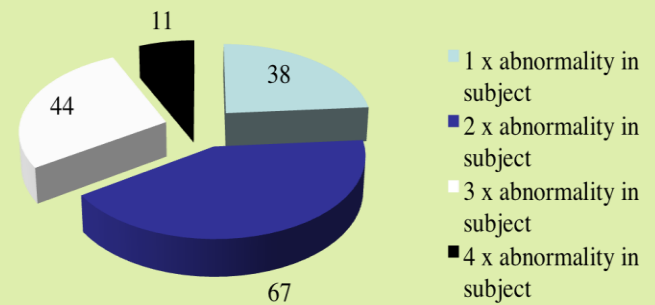
The Women's Clinic

婦產科中心

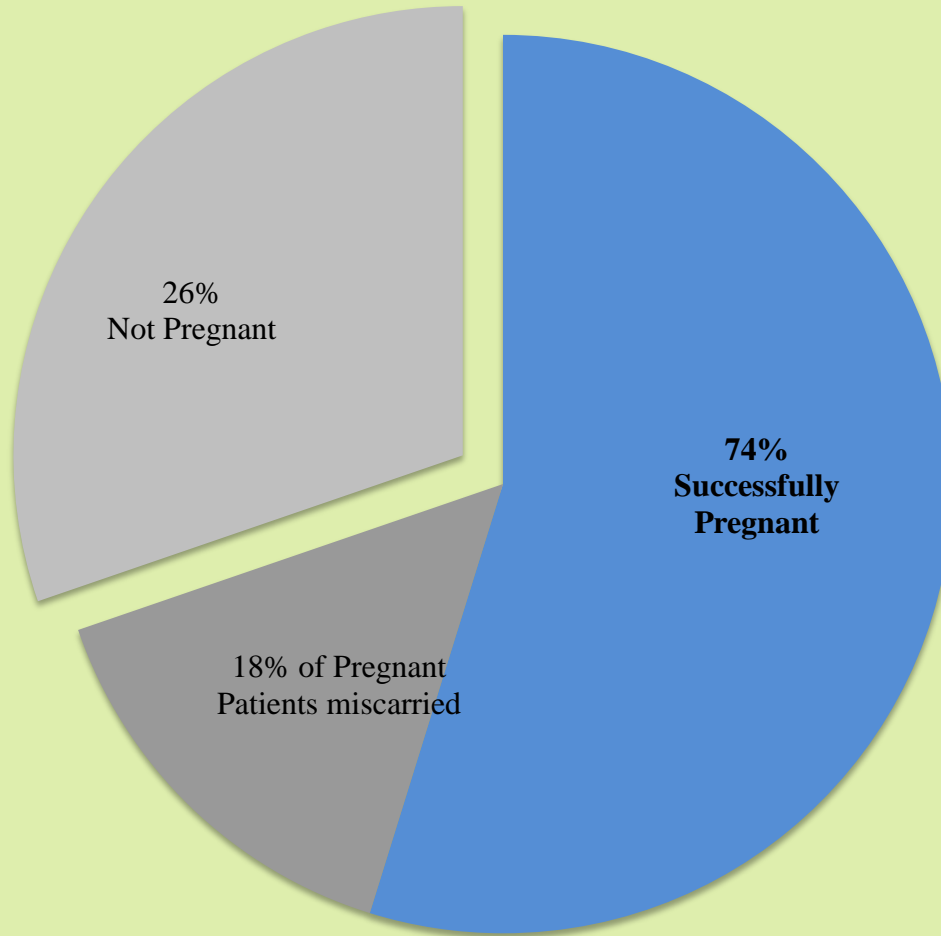
- POSITIVE IMMUNO BLOOD TEST RESULT
- NEGATIVE IMMUNO BLOOD TEST RESULT



N=134



Patients with Abnormal Blood Tests who received treatment N=117



The Women's Clinic

婦產科中心

Treatment

- All patients were treated with prednisolone from the time when follicles respond
- IVIG infusion, 40mg, given 1 week prior to egg collection, and then at embryo transfer
- Low dose aspirin given the next day of egg collection, as well as clexane, 40mg sc qd
- Luteal support was by suppository and IMI Progesterone



The Women's Clinic

婦產科中心

Treatment

- Progesterone, PT, APTT monitored regularly
- Another dose of IVIG at time of first (6 wk) ultrasound, and if FHM observed; and then 2 weeks later
- All medication tapered off after 9 weeks and when fetus appeared normal
- Patients homozygous for PAI-I pretreated with metformin from 1 cycle prior to IVF, and continued to 10 weeks



The Women's Clinic

婦產科中心

Treatment

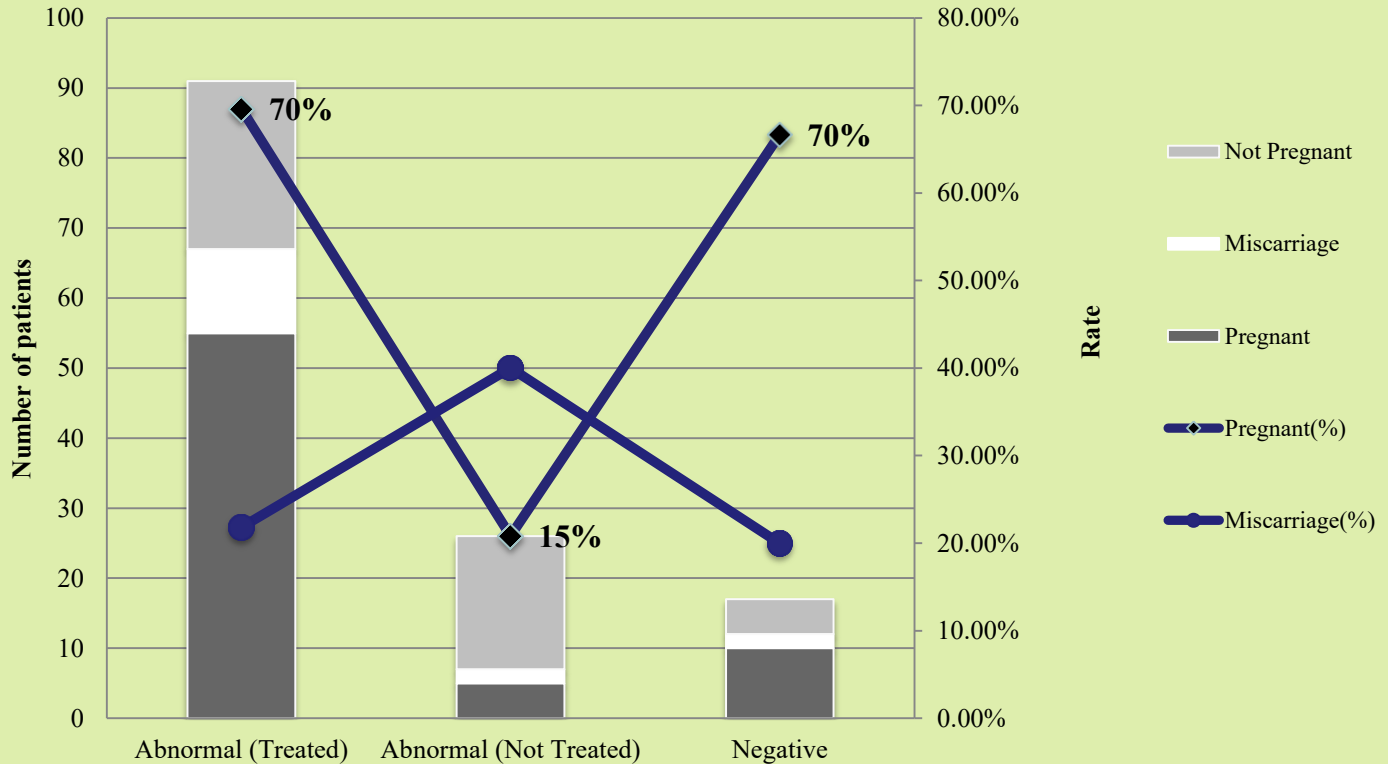
- Beginning of 2013, started using intralipid 300ML 10% infusion
- Schedule of infusion the same as IVIG
- ** Same treatment for FET which is usually done using estradiol (oral) for preparing the endometrium, and HCG trigger 2 days before starting progesterone



The Women's Clinic

婦產科中心

Pregnancy Outcome and Rate Comparison



In the three groups, total 134 pts: 91 treated, 26 untreated 17 normal
 Pregnancy rates of 71% 15% 65%
 Miscarriage rate 18%, 50% (P<0.05) and 16.6%



The Women's Clinic

婦產科中心

Action of IVIg

- Intravenous Immunoglobulin
 - To suppress elevated circulating levels of NK cells and NK cell killing activity and embryotoxins
 - To suppress elevated levels of antiphospholipid antibodies and antithyroid antibodies
 - To enhance regulatory T cell activity, suppress B cells and the production of auto antibodies
 - Activate Fc receptors, binding of complements by Fc component of IgG, adds protective function of the immune system
- Coulam CB Amer J Reprod Immunol 1998
- Sewell SAC Immunology 2002



The Women's Clinic

婦產科中心

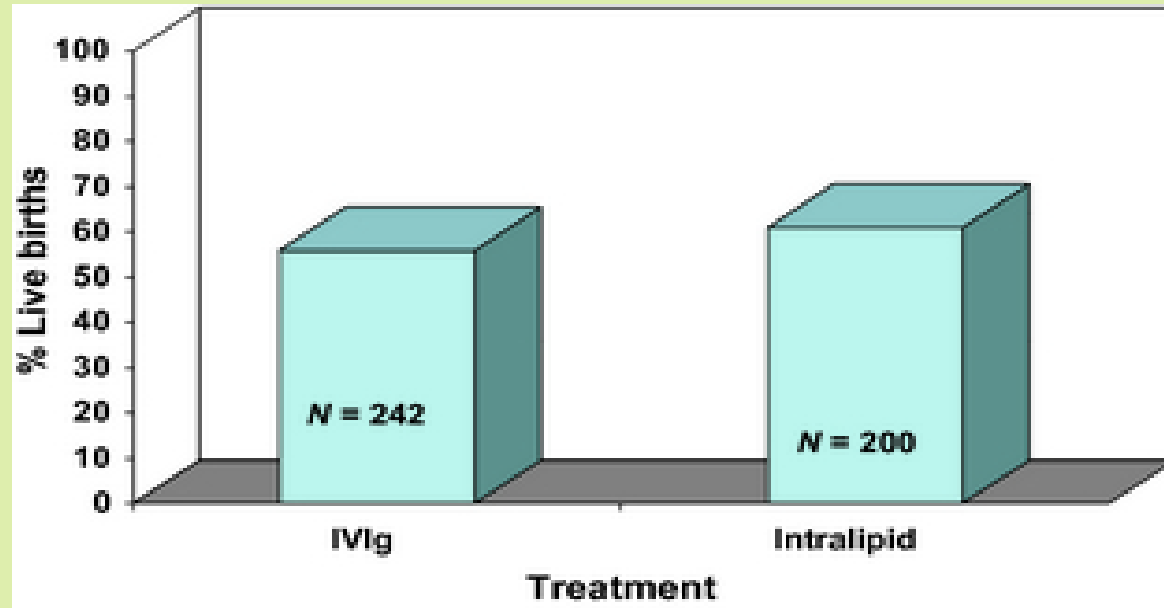


The Women's Clinic

婦產科中心

- **Intralipid:**
- **Suppresses NK cytotoxicity** Mayer K J Immunol 2003
- **Suppresses pro-inflammatory cytokine generation**
- Krog J Acta Anesthesiol Scan 2002, Inoue H Eur J Immuno 2002
- **As effective as IVIG on NK cells**
- **in vitro** Kwak J Amer J Reprod Immuno 1996
- **in vivo** Roumen GR Amer J Reprod Immuno 2008
- **Effective through fatty acid activated receptors?**
- **Peroxisome proliferative activating receptors PPAR**
- **CDI receptors**
- **G protein coupled receptors**
- Khan SA J Nutr Biochem 2003
- **Duration of Action:**
- **78% showed suppression 1 wk 1st infusion**
- **20% showed suppression after 2 wks after 2nd infusion**
- **47/50 (95%) effective 4-9 weeks**
- RoussevRG Coulam CB Amer J Reprod Immuno 2008

Does Immunotherapy for Treatment of Reproductive Failure Enhance Live Births?



Comparison of live birth rates of women with a history of reproductive failure and elevated NK cell cytotoxicity treated with intralipid and IVIg.

Age and indication matched

Coulam CB Amer J Reprod Imm 2012

American Journal of Reproductive Immunology

Volume 67, Issue 4, pages 296-304, 16 FEB 2012 DOI:

10.1111/j.1600-0897.2012.01111.x

<http://onlinelibrary.wiley.com/doi/10.1111/j.1600-0897.2012.01111.x/full#aji1111-fig-0004>



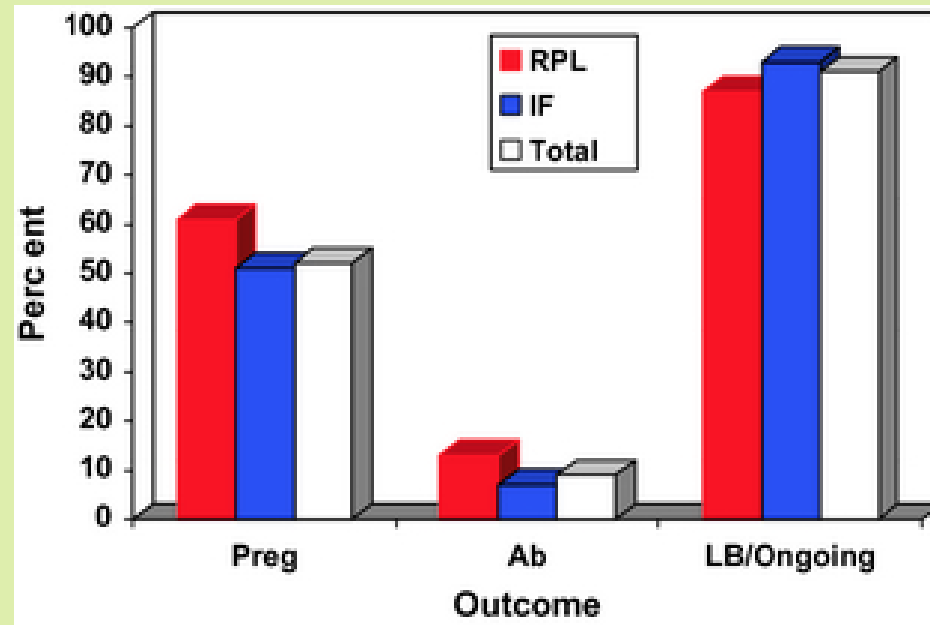
The Women's Clinic

婦產科中心



The Women's Clinic

婦產科中心



Pregnancy rates, abortion rates, and livebirth/ongoing pregnancy rates beyond the first trimester among women experiencing recurrent pregnancy loss and recurrent implantation failure who had elevated NK cell activity and who were treated with intralipid

Coulam CB Amer J Reprod Imm 2012

Single abnormal – TH1/TH2

Number of patients with just raised TH1/TH2: 4 (4/91)

All tests in panel negative and normal

Treatment with Humira alone once per week from 10 days before transfer

Treatment cycles: 5

Pregnancies: 4 (1x biochemical) SO PR 3/5 = 60%

Deliveries: 3 (Live birth rate 60%)

Reports of clinical trials with immunosuppressive oral medications:

Tacrolimus (Prograf) 60% LBR vs 0%, miscarriage 6.3%

Koji N Amer J Reprod Immunol 2012

2 Trials with Cyclosporin in China presented not published, similar successes

Thus single drug treatment for single defect possible

both Humira and Prograf proven embryo and fetal safe



The Women's Clinic

婦產科中心

Single Abnormal –NK cells



The Women's Clinic

婦產科中心

- Not common as usually accompanied by TH1/TH2 abnormal.
- 3/91 cases showed raised NK cytotoxicity only
- Treated with intralipid, clexane and aspirin
- 1 pregnancy and 1 live birth

PGS RIF and ImmunoTherapy

2 patients had PGD for feto-sexing outside HK previously

Both had multiple implantation without success (one with 5 – 8 embryos; one with 3 - 7 embryos)

Came to see me, proved to have multiple positive immunological tests.

One premature menopause at 44 with one embryo left, the other 40 with 3 embryos left

Both received immuno-modulatory treatment, and both got pregnant, delivered singleton and twins – 3 male babies

PGS available in HK 2015

20 more 'immuno-compromised' RIF pts did PGS and immunotherapy

14 pregnant (under 40 8/11 73%)

(over 40 5/8 62.5%)

1 miscarried

PGS/PGD has better results screened out abnormal fetuses but proof that PGS alone cannot overcome rejection



The Women's Clinic

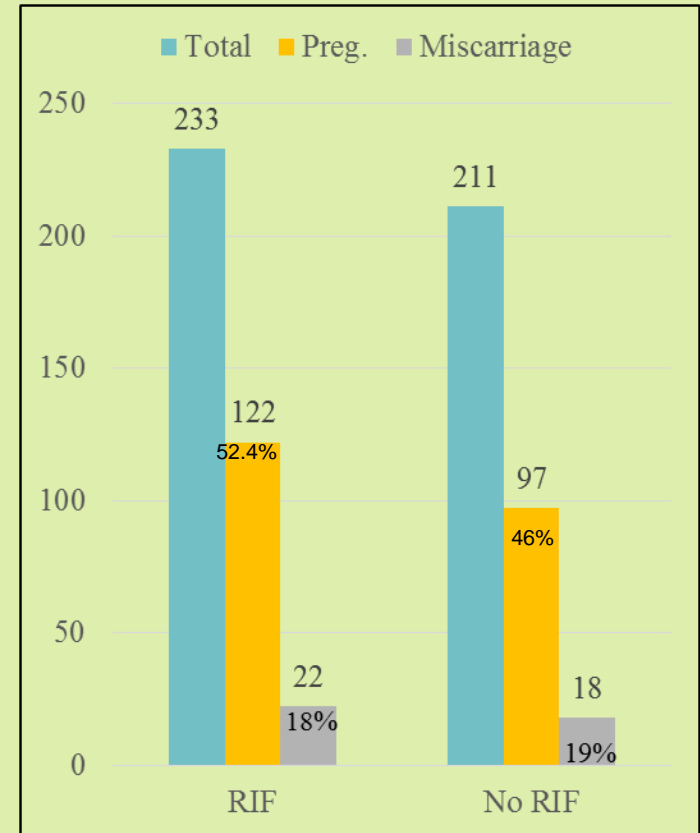
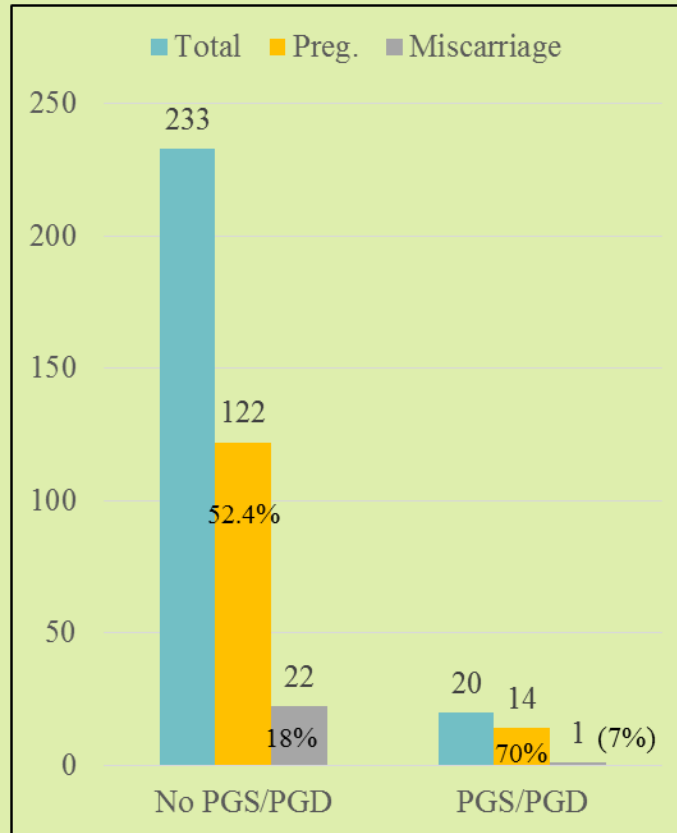
婦產科中心

Updated Results



The Women's Clinic

婦產科中心



Why When What and How



The Women's Clinic

婦產科中心

- Why is immunological causes for implantation failure known by most but accepted by so few? Why do we have to “pour” in drugs instead of rationalisation?
- When should we use the different drugs and for how long? When will we know whether specific drugs can be selectively use?
- When do we use different protocols effectively?

Why When What and How

- What is the specific mechanism of action, and what we can do to categorise and design specific tests and treatment?
- How can we obtain enough data to convince clinicians to face this problem? It may be 10% of IVF patients, but 80% of RIF patients. How can we collect enough data so the a more logical algorithm for ?different diseases be treated



The Women's Clinic

婦產科中心

Conclusions

- Our experience with a **structured approach** after identifying abhorrent immunological response has shown good and reproducible results.
- With all the variables small number “RCT” meaningless.
- We need more collaborative study to get bigger data – controlled prospective study, multicenter and multinational will give results
- Categorising and correlating with clinical findings and results will be the goal and a logical and well supported algorithm for all patients can be found



The Women's Clinic

婦產科中心

Conclusion

Human reproduction is inefficient, implantation rate is still low.

Causes are multiple and interlinked

Role of inflammation and implantation as an inflammation process may be the cause of “unexplained” implantation failure

Immunotherapy works but more needs to be done to clarify the specific relationship between different findings and clinical entities

PGS alone will not change immuno-tolerance, and not the answer

RCT's controversial; Meta-analysis small number statistic display

Some of these should be better and more critically defined

What is needed is collective and collaborative protocol of treatment



The Women's Clinic

婦產科中心

Acknowledgement

My patients who made me a believer, looked into this enigma,
and then the courage to try when nothing else worked

The Rosalind Franklin Laboratory for supporting our requests

My Team: Dr Alexander Doo and Dr Bernard Chan for clinical
collaboration, and their intellectual stimulation

Miss Freda Tang and her embryology team

Our nurses

Miss Joanne Hung, and Miss Christina Ngai for help in preparing
data and slides



The Women's Clinic

婦產科中心



The Women's Clinic

婦產科中心

Alan Beer

- Alan Beer 1937-2006
- U Penn, U Texas Southwestern, U Michigan, U Chicago Med School
- Pioneer in Immunological response in pregnancy
- Introduced the concept of embryo implantation as transplant response
- Continuous research effort in immunology as factor in placentation, pre-eclampsia, placental growth, IUGR,
- Role of anti-phospholipid antibodies, NK cells, Antipaternal cytotoxic antibodies, anti nuclear antibodies, T-cells, cytokines
- “American” side of lymphocyte infusion treatment from husband (Mulbry, Reagan UK) circa 1988 – 1998, Banned in 2003)



The Women's Clinic

婦產科中心

Alan Beer

1987 joined U. Chicago :

reproductive loss – early and late abortions, premature labours, and implantation failures

1990 onwards: A Beer, J Kwak, K, Beaman, C Coulam

- laboratory Investigation a panel of specific tests
 - application of treatment IVIG or Intralipid, with steroid and heparin and Aspirin
 - This immuno-modulatory treatment is the cumulative result of his vision and his Chicago Team
-
- Over 150 citations journals publications and books with A Beer
 - Thousands journals publication on this subject



The Women's Clinic

婦產科中心

IMPLANTATION FAILURE

- Failure to achieve a pregnancy after >5 transfers of at least 10 good quality embryos
- Nowadays, more than 3 transfers is indication for investigations



The Women's Clinic

婦產科中心



The Women's Clinic

婦產科中心

RECURRENT IMPLANTATION FAILURE

- Gamete And Embryo Factors
- Endometrial Factors
- Obesity
- Thyroid
- Uterine Factors
- Stimulation, Culture, Transfer
- Immunological Factors (5-18%)

Molecules taking part in the dialogue

Hormones

Cytokines/Chemokines

Integrins

Growth Factors

Enzymes/proteases



The Women's Clinic

婦產科中心

Cellular factors playing part in the dialogue

- Lymphocytes
- NK cells:
 - role of pNK cells
 - role of uNK cells ? Is this more important
- NK cells induced cytokine changes
 - CD +56, +19, +7
- Treg cells
- T Helper cells and their induced cytokines

- Endometrium



The Women's Clinic

婦產科中心

Role of Plasma factors in the dialogue

- Antiphospholipid antibodies
- Autoimmune antibodies
 - ANA, LE, Antithyroid
- Interferon
- Interleukins
- Plasminogen Activator Inhibitor
- Tumour Necrotising Factor



The Women's Clinic

婦產科中心

Role of the immune system in pregnancy

- Pregnancy is a state of immunologic tolerance
- In a successful pregnancy:
 - Regulatory T cells increased, suppressing T cell activation
 - TH2 response - predominantly secrete anti-inflammatory cytokines promotes implantation
 - TH1 response - increase pro-inflammatory cytokines promotes rejection





The Women's Clinic
婦產科中心

| | No of positive cases | Success cases | Successful rate (in percentage) |
|---------------|----------------------|---------------|---------------------------------|
| ANA | 8 | 7 | 87.5% |
| NK cell | 18 | 11 | 61% |
| B1 cell | 23 | 13 | 57% |
| APA | 17 | 8 | 47% |
| TH1:Th2 ratio | 24 | 15 | 63% |