Intravenous Immunoglobulin for Repeated

IVF Failure and

Unexplained Infertility

Michael R. Virro, American Journal of
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INTRODUCTION

Repeated in vitro fertilization (IVF) failure

- Canadian ART Registry (CARTR statistics) → 2010 IVF success rates:
  - Per cycle started with 30% Overall live birth rate (3160 live births of 10,532 cycles started)
  - Failures → 1/6 'unexplained'

'Unexplained' failures

- Immunologic basis
  - Pregnancy: An unique immunologic challenge to the maternal immune system
  - Fetus: A hemi−allograft (fetal & maternal tissues are intimately associated)
- Immunotherapy
  - Esp. Intravenous immunoglobulin (IVIG)
  - Direct modify immune/inflammatory response → ↑ immunologic tolerance & pregnancy success

Candidacy for immunotherapy

- Suggested by altered immune function:
  - Preconception blood natural killer cell cytotoxicity
  - Proportion of peripheral blood CD56+ CD3– cells
  - Th1/Th2 ratio
  - Circulating level of regulatory T cells (Tregs)

1. *Stephenson et al.*

- Women with idiopathic 2nd recurrent miscarriages → treated with IVIG vs. placebo

→Impproved pregnancy outcome

→Magnitude of the improvement: Insufficient to exclude the role of chance

→Ended the study before the planned sample size had been accrued and explicitly

→Excluded: Specific immune test abnormalities

- Control group： ↑Spontaneous rate of success → than 2 other studies (significant % of the patients included immune abnormalities)
- IVIG-treated group： Slightly ↑ success rates → No statistical significance

☆The benefit of IVIG(*Van den Heuvel et al.*)：

   ☆Restricted to patients with autoimmunity or

   ☆↑blood CD3+ CD56+(↓to normal with IVIG therapy)

1. *Winger et al.*

- Preconception Th$_1$/Th$_2$ and/or NK elevation → predicted IVIG benefit in IVF

- If the treatment actually corrected the abnormal Th$_1$/Th$_2$ ratio → A successful live birth was more likely

∴ Patients with **repeated failures** despite 'optimal' IVF

→May with immunologic abnormality causing failure

→May be amenable to IVIG therapy

IVIG therapy

- For patients with pregnancy failure: off-label indication

- Initial use: 1981, 1º & 2º immunodeficiency → Ameliorate Immune thrombocytopenia

- Useful:

  - In disorders caused by pro-inflammatory cellular immunity: Kawasaki disease, dermatomyositis, multiple sclerosis, graft versus host disease

  - In haematopoietic stem cell transplantation to prevent graft versus host disease

IFFS 16th World Congress on Fertility and Sterility in San Francisco, 1998, Virro

- Randomized, prospective study

- Comparing couples undergoing their 1st IVF cycle (n = 31 each group)

- Half of the patients received IVIG at the time of egg retrieval (half did not)

  - IVF pregnancy rate: 70% vs. 50% (No statistical significance at this sample size)

  - Live birth rate: Higher in those receiving IVIG

Mechanisms of immunosuppressive activity of IVIG therapy

1. Effect of IVIG on FcγRs

    → Down-regulate: activating receptors (FcγRI, FcγIII) on human monocytes in Kawasaki's disease patients

   → Up-regulate: inhibiting receptor (FcγRIIB) in various animal models

2. Anti-inflammatory activity of IVIG

→ Specific glycosylation at asparagine 297

    → An amino acid residue

    → In the Fc portion of the molecule (FcγR interacting portion of immunoglobulin)

1. Terminal sialic acid $--\alpha2-6$ **glycosidic linkage**-- Penultimate galactose
   - In an IVIG preparation accounts
   - On only a small % of immunoglobulin molecules
   - Much of IVIGs suppressive activity of an IVIG preparation
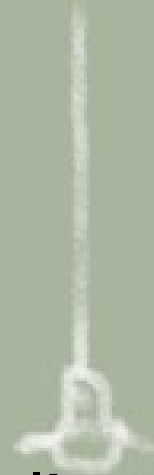- Pregnancy: Associated with ↑**Sialylated IgG** antibodies
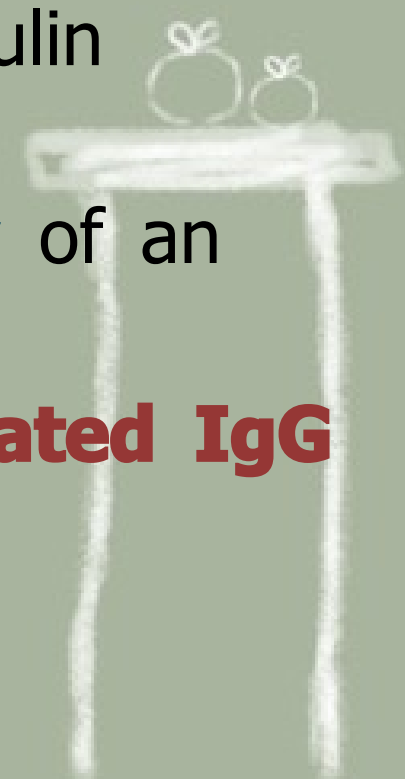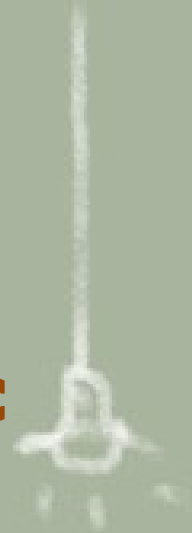
1. Suppression of NK-type cell activity

   − 1/3 due to **CD200** : A tolerance signaling &
   Treg-promoting molecule

   ☆↑ NK levels in peripheral blood:
     Specifically linked to Miscarriage of
     karyotype normal embryos

In this retrospective study

- Multiple(2) prior IVF failures ±'unexplained' infertility

➔ IVIG on the day of egg retrieval during their IVF cycle

- In the absence of immunologic testing
  - Patients with more 'unexplained' failures → ↑ incidence of immunological abnormalities
  - 2~3 vs. ≥ 4 consecutive IVF failures

- Subsequent success rates: Compared with published success rates from the Canadian database

- Live birth rates per cycle (LBR/c): Can be misleading
- Live birth rates per embryo transfer (LBR/e)
  - Karyotype abnormal oocytes → High frequently lead to failure
  - Optimal expected success rate for LBR/e: 50%
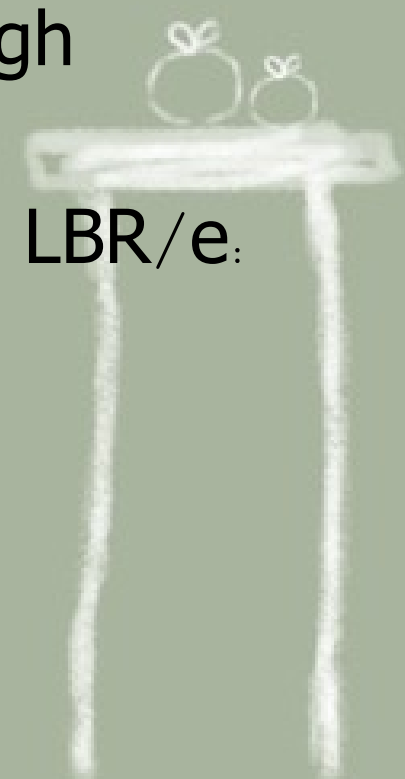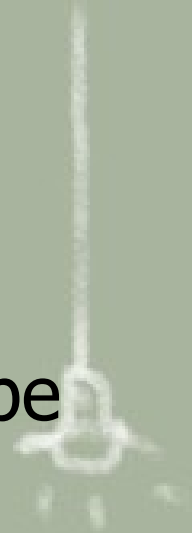
MATERIALS AND METHODS

Patient selection

- January 1999 ~ December 2011
- Hx of repeat IVF failure &/or "unexplained" infertility
- Offered IVIG for their next IVF cycle
- At the Markham Fertility Centre in Ontario, Canada

  ➔229 eligible treatment cycles were included

  ➔Donor egg and frozen egg cycles were excluded

Protocol

1. Stimulation cycle: Determined by their previous response to previous IVF protocols
2. *Agonist protocol:
Lupron 0.1ml (starting day 21 for 14 days)
*Antagonist protocol:
Orgalutron starting on their 5th day of stimulation
3. *FSH alone (Puregon or Gonal-F)
*Combination of FSH and Menopur (75 IU FSH activity + 75 IU LH activity ← 95% LH activity from HCG)
4. HCG 10,000 units: 2-3 lead follicles → 1.8 cm

Natural Killer Cell Assay

- Cytotoxicity (50:1 effector: target cell killing ratio)

  – NK sensitive cell line K–562 = Target cell

  →Co–cultured with

  – Peripheral blood mononuclear cells (PBMC)

  →% of Target cells killed by effector NK cells

  →Dete

$$\% \text{ Cytotoxicity} = \frac{\text{Killed Target Cells} = R2}{\text{Killed Target Cells} + \text{Live Target Cells} = R2 + R1} * 100$$

IVIG Therapy

- 400 mg/km body weight On the day of egg retrieval

- Natural Killer Cell Assay cytotoxicity result $>15\%$
  $\pm$ CD$56 > 12\%$ $\pm$ Positive pregnancy test
  $\rightarrow$ Additional IVIG (43 cases)

- During the 1st trimester of pregnancy
  $\rightarrow$ Repeated monthly $\%$ CD56^+ cell $\&$ NK cytotoxicity assessment
  $\rightarrow$ Remained $\uparrow$
  $\rightarrow$ Additional 25 g IVIG

Addition Immunotherapy

- 12% (28/229), Based on preconception testing
- Anti-tumor necrosis factor alpha (TNFa) therapy

➔ TNF-α : IL-10 >30.6 ± IFN-γ : IL-10 >20.5

➔ 2 x injections of Humira (3rd TNF inhibitor) 40 mg SC

  ➔ Initiated: 30-120 days before starting a cycle of conception

  ➔ Given 2 weeks apart

  ➔ Discontinued prior to the onset of cycle stimulation

lymphocyte immunization therapy (LIT)

- Anticoagulants, Clexane® 30 mg QD (Started preconception, within 6 months preconception)

- Acquired thrombophilia:

  - (+) Antiphospholipid antibody, 1:50

  - ≥1 : Cardiolipin, Serine, Ethanolamine, Glycerol, Inositol, Phosphatiditic acid *(IgM, IgG, or IgA)*

- Inherited thrombophilia:
  - (+) Following genetic mutations ≥ 1 :
    - Heterozygous or homozygous factor V Leiden $R_{506}Q$
    - Prothrombin $G_{20210}A$
    - Plasminogen activator inhibitor $4G/5G$
    - Homozygous methylene tetrahydrofolate reductase (MTHFR) $C_{677}T$
    - Compound heterozygous MTHFR $C_{677}T/A_{1298}C$

IVF Procedure

- Fresh IVF cycles only

- Negative IVF outcome:
Day 12 post-transfer → Serum beta HCG <10

- Live birth:
Delivery of a live-born child

- Live birth rate per embryo (LBR/embryo):
Numbers of babies born per embryo transferred
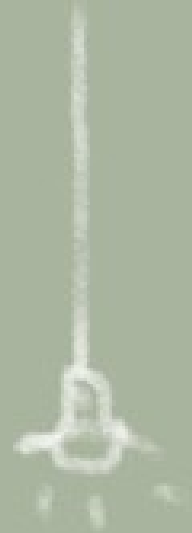
Statistical Analysis

- Success rates:
  - Fisher's exact test
  - T-test
- Graphpad Software®, La Jolla, CA, USA

RESULTS

General characteristics

	Total	<35 years	35–39 years	≥40 years	≥4 prior in vitro fertilization (IVF) failures
Number of cycles	229	115	90	24	78
Baseline characteristics (Mean ± S.D.)					
Maternal age (years)	34.6 ± 3.8	31.5 ± 2.4	37.0 ± 1.4	40.5 ± 0.7	34.8 ± 3.7
Duration of infertility (years)	3.8 ± 2.7	3.6 ± 2.3	4.1 ± 3.1	3.8 ± 3.2	4.3 ± 3.1
No. prior IVF failures	3.3 ± 2.1	3.1 ± 1.8	3.6 ± 2.4	3.1 ± 1.9	5.2 ± 1.3

IVIG treated cycle success rates

	Total	<35 years	35–39 years	≥40 years	≥4 prior in vitro fertilization (IVF) failures
IVIG treated cycle success rates					
Pregnancy rate	60.3% (138/229)	69% (79/115)	56% (50/90)	38% (9/24)	51% (40/78)
Live birth rate	40.2%** (92/229)	47% (54/115)	38% (34/90)	17% (4/24)	33%* (26/78)
Live birth rate/embryo	23.3% (108/463)	27.4% (64/234)	21.9% (40/182)	8.5% (4/47)	16.6% (26/157)
For comparison: CARTR Statistics 2011 (10,532 cycles)					
Pregnancy rate	–	43.1%	33.8%	17.6%	23.4%[56] (67/286)
Live birth rate	30%**	40%	29%	12%	15.7%*[56] (45/286)

P < 0.0001
P < 0.001

	Day 3	Day 5	Single embryo	Two Embryos	2 Day 5 High-grade embryos
IVIG treated cycle success rates					
Pregnancy rate	52% (66/126)	69% (68/99)	61% (20/33)	62% (99/160)	97% (29/30)
Live birth rate	37% (47/126)	43% (43/99)	48% (16/33)	37%*** (59/160)	607% (18/30)
Live birth rate/embryo	21.0% (57/272)	19.5% (8/41)	27.4% (51/186)	25% (79/320)	41.9%% (26/62)
For comparison: CARTR Statistics 2011 (10,532 cycles)					
Pregnancy rate	37% (566/1531)	48.2% (522/1082)	34.9% (428/1225)	43.1% (1137/2639	$P = 0.001$*
Live birth rate	–	–	–	–	$P < 0.00012$**

P = 0.0008   P = 0.0001

P = 0.0001

Table II Primary Infertility Diagnosis for 229 Patients

Diagnosis	Frequency
Unexplained	27% (61/229)
Male factor	20% (45/229)
Tubal	12% (27/229)
Endometriosis	10% (23/229)
Other	32% (73/229)

	Total	<35 years	35–39 years	≥ 40 years	≥ 4 prior *in vitro* fertilization (IVF) failures
Supplementary immunotherapy					
Lymphocyte immunization therapy	9% (21/229)	6% (7/115)	11% (10/90)	17% (4/24)	5% (4/78)
Humira	11% (25/229)	9% (10/115)	11% (10/90)	21% (5/24)	4% (3/78)
Heparin	20% (46/229)	18% (21/115)	16% (14/90)	42% (10/24)	13% (10/78)

DISCUSSION

"Unexplained" Recurrent IVF failure

- ± Unexplained Infertility → IVIG → ↑Live birth rate/cycle (vs. General experience)

- Live birth rate/embryo (LBR/e)
  - Not generally reported in the CARTR database
  - This study: 41.9% ⇔ Predicted Max rate: 50%

- 2 x cohort-controlled studies: (IVIG + anticoagulants in IVF/Humira & IVIG + anticoagulants in IVF failure)
  → ↑Th1/Th2 (± ↑% NK)
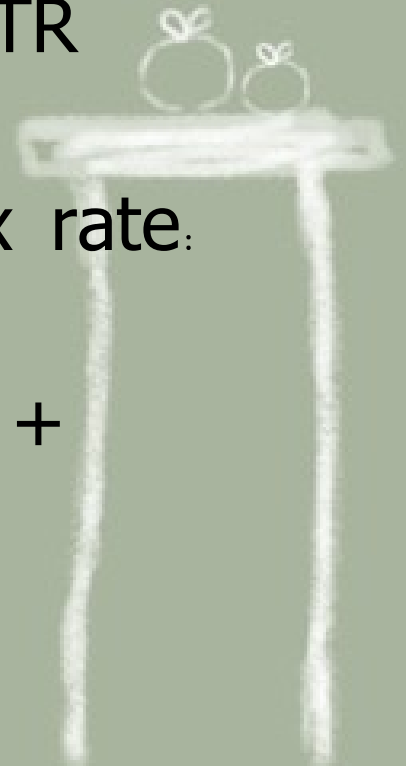
Immune parameters

- 'Unexplained' infertility ⇔ Abnormalities in several different in vitro immune parameters
  - Preconception blood natural killer cell cytotoxicity
  - Proportion of peripheral blood $CD56^+/CD3^-$ cells
  - Th1/Th2 balance
- This study:
  - Including only patients with a difficult failure Hx
  - Selected patients with these immunologic

Select patients most suitable for immunotherapy
Immunologic conditions

- History of 4 prior IVF failures *(5.2 ± 1.3)* → IVIG → ↑↑ Delivery rate

- 43 patients underwent immunologic testing

  → 28: Received additional immunotherapies

    → Pregnancy rate & delivery rate: ↑over those receiving IVIG alone

      − 74%(14/19) versus 60.3%(138/229, total)

      − 56%(11/19) versus 40.2%(92/229, total)

- Future: Optimize patient selection for treatments

Select patients most suitable for immunotherapy
Embryo grade scores

- Patients with the highest quality blastocysts transferred (≥Grade 3)

- With a history of repeat or 'unexplained' failure

  ➔IVIG

  ➔Nearly 100% pregnancy success rate "98% (30/31)"

- When non-immunologic causes have been ruled out (elimination of the poor-quality embryos)

  ➔Immunological (often 'unexplained') causes

Embryo quality

- Humira:
  - May improve embryo quality
  - Especially if administered > 60 days prior to egg harvest, during the time of folliculogenesis
- Lymphocyte Immunization Therapy:
  - Many studies support an immunomodulatory role in pregnancy

➔What specific immune abnormalities benefit from LIT?

➔What testing should be performed to identify

Single-embryo transfers

- Commonly use high–quality embryos
- ➔ Permits acceptable take–home baby rates with ↓ net embryos being transferred
- ➔ IVIG(+) vs. CARTR pregnancy rates in cycles IVIG(–):
  Almost doubling of the single–embryo pregnancy rate 61%(20/33) vs 34.9% (428/1225)

	Day 3	Day 5	Single embryo	Two Embryos	2 Day 5 High-grade embryos
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Live birth rate	–	–	–	–	$P < 0.00012**$

Single-embryo transfers

- Live birth rate:

  - Single–embryo transfer > Two embryo transfers

- Singleton births:

  - Two ⇔ Single–embryo transfer →
    Delivered:
    68% (40/59) ⇔ 100% (20/20)

- IVIG + single–embryo transfers in patients with 'unexplained' infertility → ↓Multiple pregnancy rates

 - ↓Cost of IVF (Treatable Immunological

Summary

- IVIG

  - May be a useful treatment option for patients with previous IVF failure and/or unexplained infertility

  - ↑ IVF success rates in women with multiple prior IVF failures and immunologic infertility

  - ↓ Multiple pregnancy rates

  - ↑ 'Takehome baby' rates

In the future

- Further investigate testing protocols that optimize <u>patient selection for immunologic treatments</u>

- Larger prospective controlled studies
  → Confirm these findings with particular attention paid to treatment subgroups
  → Testing required to determine if correction of testing abnormalities predicts a higher success rate
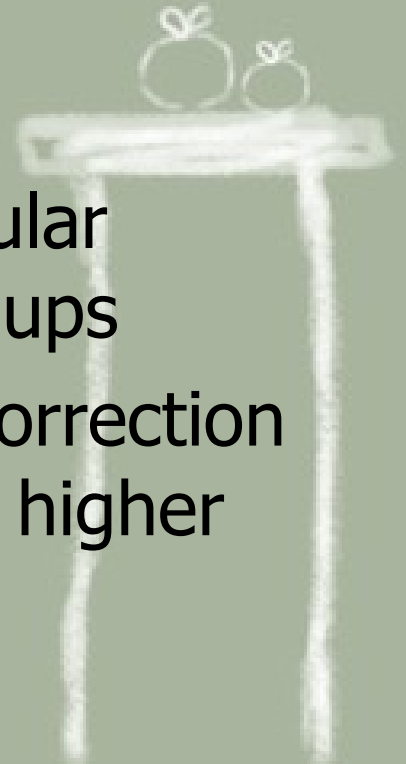
Thank your for listening

Further Discussion