Tacrolimus And RIF

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Implantation failure : situation in which a **good-quality embryo** has been transferred into the uterine cavity but **has failed** to establish a pregnancy .

Crude estimation based on the clinic's pregnancy rates

It is recognized that carrying our individual calculations per patients may not always be feasible. An alternative approach could be to make an estimation, based on the clinic's pregnancy rate for specific patient groups.
For the example presented in the table, published pregnancy data were used.
While there will be some women who might be expected to have a 60 % chance of implantation after just embryo transfer, the minimum number for identifying implantation failure to be 'recurrent' should be two'.

	Maternal age	Implantation rate / pregnancy rate[1]	Cumulative likelihood of implantation for each embryo transfer (embryos of unknown euploidy)						RIF THRESHOLD of >60%
			FIRST ET (n=1)	SECOND ET (n=2)	THIRD ET (n=3)	FOURTH ET (n=4)	FIFTH ET (n=5)	SIXTH ET (n=6)	
Embryos of unknown euploidy	<35	31,5	31,5	53,1	67,9	78,0	84,9	89,7	Intervene after 3 ETs
	35-39	25,9	25,9	45,1	59,3	69,9	77,7	83,4	Intervene after 4 ETs
	≥40	15	15,0	27,8	38,6	47,8	55,6	62,3	Intervene after 6 ETs
Euploid embryos	<35	68,4	68,4	90,0	96,8	99,0	99,7	99,9	Intervene after 2 ETs
	35-40	64,1	64,1	87,1	95,4	98,3	99,4	99,8	Intervene after 2 ETs
	>40	58,0	58,0	82,4	92,6	96,9	98,7	99,5	Intervene after 2 ETs

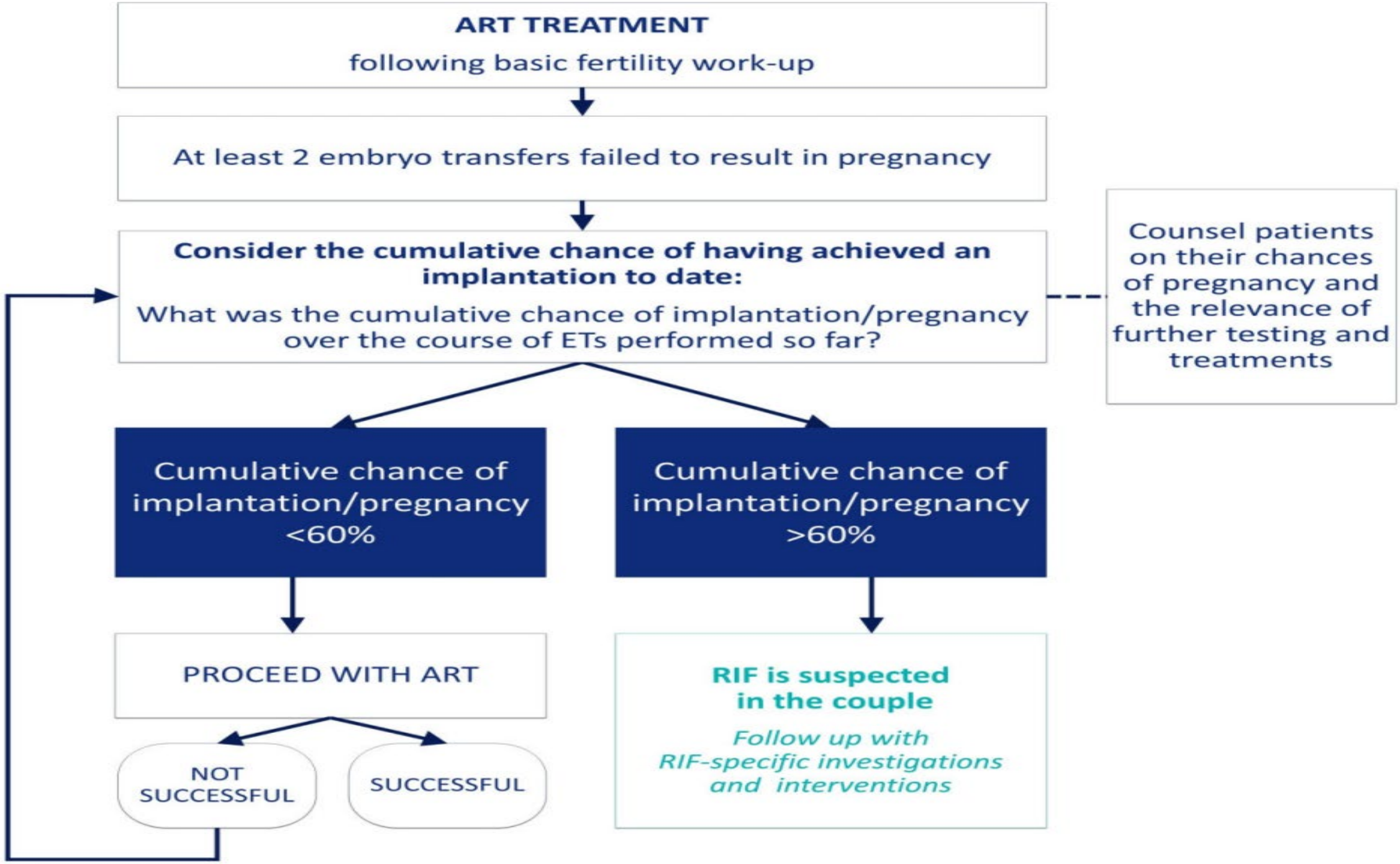
36-year-old woman who has been trying to conceive for 3 years, has damaged tubes, never been pregnant and never had IVF before. She uses her own eggs.

Estimation based on the IVFPredict calculator

With the use of the IVFPredict calculator from the Nelson and Lawlor model (ivfpredict.com), the following calculations can be made for this specific patient:

Her chance of live birth per IVF attempt is 23.8%	according to the IVFPredict tool
Her chance of pregnancy per IVF attempt is 27,6%	calculated by multiplying the LBR by 1.16 to obtain chance of pregnancy i.e., 23.8 X 1.16 = 27.6%
The chance of pregnancy is	calculated by $NP_n = (1\text{-}PR)^n$
• **47% over the course of 2 ET attempts**	$1- [(1-0.276) X (1-0.276)] = 0.47$
• **62% over the course of 3 ET attempts**	$1- [(1-0.276)^3] = 0.62$
• **72% over the course of 4 ET attempts**	$1- [(1-0.276)^4] = 0.72$
• **80% over the course of 5 ET attempts**	$1- [(1-0.276)^5] = 0.80$

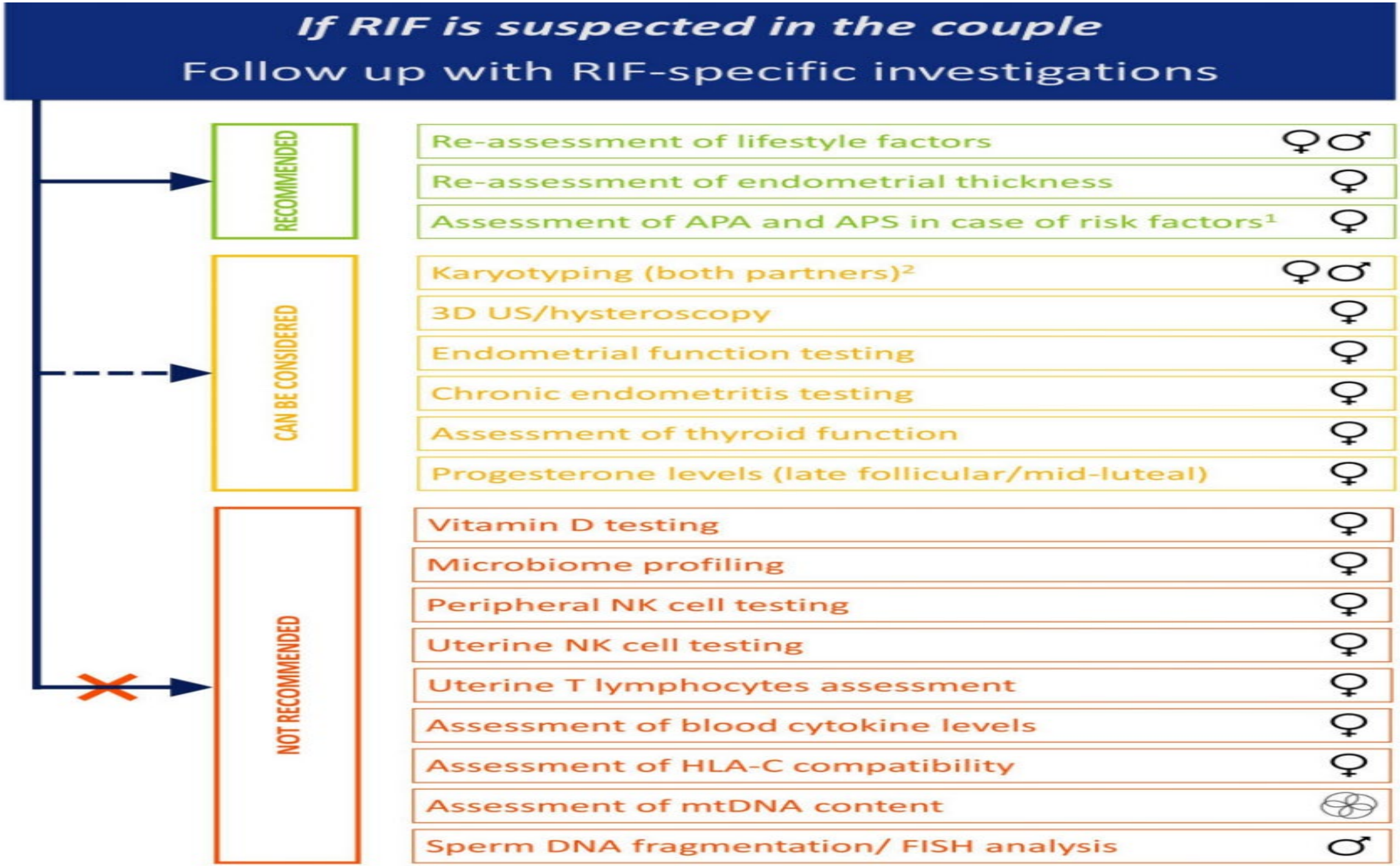
According to the threshold for RIF of >60%,
if the woman is not pregnant after 3 ETs we intervene.

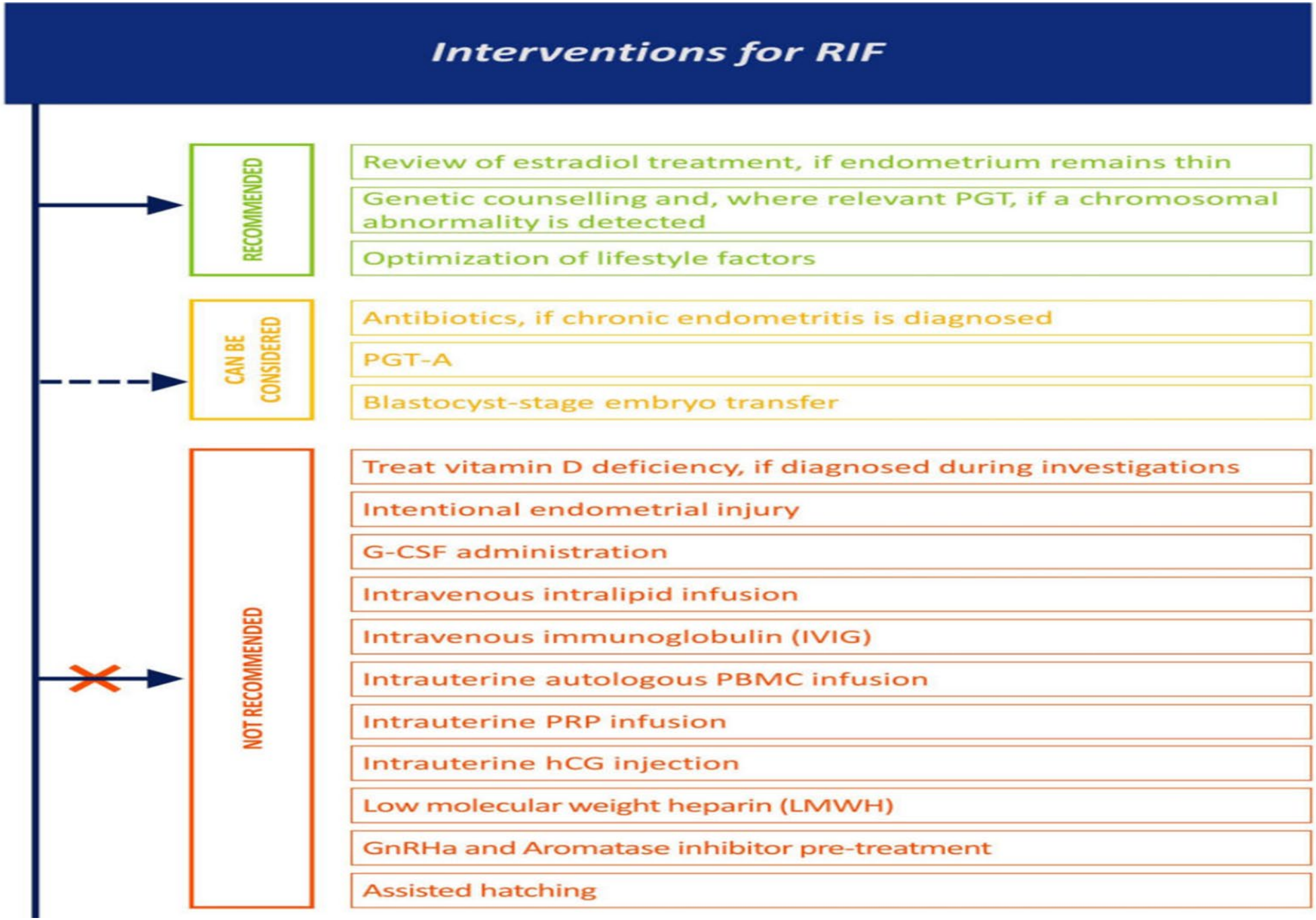


Summary: applying an individualized definition of RIF in clinical practice. This flow diagram follows the a priori condition that the p

three levels of advice for all patients with suspected RIF:

- **green**: recommended
- **orange**: can be considered
- **red**: not recommended


If RIF is suspected in the couple
Follow up with RIF-specific investigations
RECOMMENDED
Re-assessment of lifestyle factors ♀♂
Re-assessment of endometrial thickness ♀
Assessment of APA and APS in case of risk factors[1] ♀
CAN BE CONSIDERED
Karyotyping (both partners)[2] ♀♂
3D US/hysteroscopy ♀
Endometrial function testing ♀
Chronic endometritis testing ♀
Assessment of thyroid function ♀
Progesterone levels (late follicular/mid-luteal) ♀
NOT RECOMMENDED
Vitamin D testing ♀
Microbiome profiling ♀
Peripheral NK cell testing ♀
Uterine NK cell testing ♀
Uterine T lymphocytes assessment ♀
Assessment of blood cytokine levels ♀
Assessment of HLA-C compatibility ♀
Assessment of mtDNA content
Sperm DNA fragmentation/ FISH analysis ♂


Interventions for RIF
RECOMMENDED
Review of estradiol treatment, if endometrium remains thin
Genetic counselling and, where relevant PGT, if a chromosomal abnormality is detected
Optimization of lifestyle factors
CAN BE CONSIDERED
Antibiotics, if chronic endometritis is diagnosed
PGT-A
Blastocyst-stage embryo transfer
NOT RECOMMENDED
Treat vitamin D deficiency, if diagnosed during investigations
Intentional endometrial injury
G-CSF administration
Intravenous intralipid infusion
Intravenous immunoglobulin (IVIG)
Intrauterine autologous PBMC infusion
Intrauterine PRP infusion
Intrauterine hCG injection
Low molecular weight heparin (LMWH)
GnRHa and Aromatase inhibitor pre-treatment
Assisted hatching

successful implantation is determined by a multitude of factors:

- **female-related factors :** age, hormonal levels, endometrial and uterine status
- **embryo-related factors** : embryonic cleavage speed, euploidy
- **male factors** : genetic disorders
- **external factors** : the performance of the laboratory and clinic, transfer policies, and legal restrictions.

RIF may be attributed to a decrease in endometrial receptivity, which can result from dysregulation of the endometrial immune profile . addressing this mechanism could potentially enhance the chances of successful pregnancies in patients with RIF.

Maternal immune tolerance :

- window of implantation (**WOI**) :substantial influx of immune cells to shelter the embryo from refusal .

- Maternal immune tolerance during pregnancy is maintained by the delicate interplay of **cytokine** signals, which is facilitated by immune cells such as **T-helper cells** (Th1, Th2, and Th17) and **regulatory T-cells** (Treg) .

Th1

- role in cell-mediated immunity
- producing cytokines such as IL2, TNF-α, IFN-γ
- trigger **inflammation**
- there is a dominance of Th1 in peri-implantation phase .
- regulated TH1 immunity is advantageous for the invading trophoblasts rather than causing harm.

Th2

- humoral responses
- produce **anti-inflammatory**
- cytokines like IL-4 and IL10.

Following successful implantation: the immune response of the endometrial lining transitions from a cell-mediated to a humoral response.

if there is an unequal distribution of Th1 and Th2 cells and their cytokines, it can lead to RIF.

maternal-fetal immune abnormalities

→ *uteroplacental dysfunction*, *insufficient fetal immune tolerance*

→ *1-fetal rejection*,
2-obstetrical complications, such as unexplained pregnancy loss and preeclampsia

evaluation of immunologic parameters:

- especially in women with a history of pregnancy loss or implantation failure.
- would help clinicians to manage the disorder and prevent next unfavorable pregnancy outcomes.
- including cellular and humoral immunity assessment (T and B lymphocyte, T helper subtypes, NK cells, cytokines, and autoantibodies).

Given the lack of a clear relationship between immunephenotypes and ART outcomes, the use of immunological testing in the general ART population cannot be recommended .

several **immunomodulatory approaches** have been introduced to modulate the abnormal immunologic responses in patients who experience reproduction failure, especially those diagnosed with immunologic basis.

Tacrolimus

- immunosuppressant agent.
- calcineurin inhibitor.
- widely used in solid organ transplant recipients.
- **primary effect** of tacrolimus is its involvement in the direct inhibition of activated NK/NKT and Th1 cells.inhibits antigen-induced lymphocytic proliferation, cytotoxic T-cell formation, IL-2 receptor expression, and the production of IL-2 and IFN-γ

- is expected to :
  - endometrial maturation during the implantation window
  - **promote tolerance** to early embryos after ART by modulating the immunological environment of the preimplantation endometrium.
  - in Th1- dominant immune states: might have a **preventive effect** on the pathological conditions suggestive of fetal rejection and uteroplacental dysfunction, including unexplained or preeclamptic stillbirth

Personalized immunotherapy in RIF patients with different endometrial immune profiles

eshre2024-374

What is known already:

Personalized medicine

- in the area of **infertility** is in its early stages.
- considering the promising results of personalization of treatment in other disorders, including **cancer**
- it is expected to have acceptable results in infertility as well.

Given that **one of the key factors** that affects the infertility of RIF patients is the imbalance of the endometrial immune system, **efforts** have been made to correct this disorder to increase the chances of fertility.

Study design, size, duration: The study selected **267 RIF patients** who were referred to Valiasr Hospital in **Tabriz** from Aug 2020-Sep 2023

267 RIF patients(based on the **endometrial immune system profiles**, by the gene expression level of *IL-18*, *IL-15, TWEAK*, *Fn-14*, and *CD56*)Divided into five groups:

- unclassified RIF patients (n=89) =who underwent routine IVF/ET cycles **without immunotherapy**
- normal and balanced endometrial immune system profile (n=63) =received **no immunotherapy**
- high (n=53) profiles of endometrial immune system =received **vaginal probiotics** and **Tacrolimus**
- low (n=38) profiles of endometrial immune system =**vaginal probiotics** and **lymphocyte therapy** were used.
- mixed (n=24) profiles of endometrial immune system = **vaginal probiotics**, **Tacrolimus**, and **lymphocyte therapy** were used.

Main results and the role of chance:

The primary and secondary endpoints of this study were **clinical pregnancy** and **delivery of a live born infant**, respectively.

- unclassified :25.8% and 23.5%.

- Balanced group :28.5% and 25.3%

- **it was significantly higher in:**
  - High (56.6%, 54.7%),
  - Low (47.3%, 44.7%)
  - Mixed groups (37.5%, 29.1%)

According to the obtained results, it was found that the appropriate immunotherapy treatment based on the endometrial immune activity profiles in RIF patients has significantly increased the effective implantation rate in the IVF/ ET cycles (P<0.05).

Limitations, reasons for caution:

- It is necessary to examine a larger number of patients
- Further work is needed to explore other parameters that are affected by *endometrial immune system* disorder and that can help *classify RIF patients* and *recommend the most effective treatment.*

Wider implications of the findings:

- ❑ For RIF patients, accurate identification of the cause of the disorder can be very effective for choosing the best treatment method.
- ❑ Personalized immunotherapy for RIF patients with immunologically abnormalities can lead to very promising and acceptable results while having few complications.

Tacrolimus treatment
significantly improved IVF outcomes
with euploid embryo transfer in women
with RIF and elevated Th1/Th2 cell Ratios

(eshre 2024)17

What is known already:

- **Previously**, we reported that about 40% of women with RIF (≥4) had elevated Th1/Th2cell ratios (≥10.3).

The **pregnancy rate** was significantly **improved** when they were treated with **tacrolimus** [AJRI 2015].

Recently, we reported an adjusted cut-off value of the Th1/Th2 cell ratios (≥11.8) [AJRI2021] for women with RIF based on the retrospective analysis. However, the prospective study has not been performed.

Study design, size, duration:

- A prospective cohort study

- from September 2020 to November 2021(Japan)
- **569** infertile women with ＞**4 times of RIF**

- The study participants received ET with **euploid blastocysts .** All transferred blastocysts were confirmed as euploid or low-frequency mosaic by PGT for aneuploidy

- endometrial preparation for ET was made by using either **hormone replacement cycle** or **natural ovulatory cycle**.

Participants/materials, setting, methods:

- ✓ measured peripheral blood Th1/Th2 (IFN-γ/ IL-4) , Before ET in the secretory phase.
- ✓ Women who had elevated Th1/Th2 ≥10.3 received tacrolimus (2-4 mg daily). started 2 days before ET.
- ✓ the **hCG** and gestational sac (**GS**) **rates** were calculated in women with or without tacrolimus.

Main results and the role of chance:

569 RIF>4 :

174 (30.6%) women showed elevated Th1/Th2 cell ratios (≥10.3)

- 148 women received tacrolimus (Tac group)
- 26 did not receive tacrolimus (no-Tac group).

395 women served as control (Control group).

- **hCG and GS rates** of
  - Tac group were 73.0% and 64.2%, respectively

similar to

- no-Tac group (69.2% and 57.7%, respectively)
- control group (70.6% and 60.0%, respectively)

Main results and the role of chance:

569 RIF>4

123 women (21.6%) had elevated Th1/Th2 cell ratios (≥**11.8**)

- 112 received tacrolimus (Tac2 group)
- 11 did not tacrolimus (no-Tac2 group).

410 women were as a control group (Control2 group).

❖ **GS rate** of:

- Tac2 group was 67.0%

Significantly higher than that of no-Tac group (36.3%, p<0.05)

✓ Control2 group was 60.5% (P¼NS, vs Control2 group).

✓ Tacrolimus dosing data was as follows;

- ≥11.8 but <15.8 of Th1/Th2 cell ratios →2mg of tacrolimus daily
- ≥15.8 →3mg of tacrolimus.

Limitations, reasons for caution:

- The study was a controlled trial with a limited number of study population.
- the endometrial changes or peripheral immune responses **after tacrolimus** were not evaluated thoroughly.

Further study is needed for the tacrolimus effect on systematic immunenresponses and local endometrial immune milieu.

Wider implications of the findings:

- Before ET, screening for increased Th1/Th2 ratios (Cut-off value **11.8**) may significantly **improve** IVF outcomes by selecting proper candidates for tacrolimus treatment and detecting the proper tacrolimus doses.

The role of immunotherapy in in vitro fertilization: a guideline

Practice Committee of the American Society for Reproductive Medicine

American Society for Reproductive Medicine, Birmingham, Alabama

Adjuvant immunotherapy treatments in in vitro fertilization (IVF) aim to improve the outcome of assisted reproductive technology (ART) in both the general ART population as well as subgroups such as patients with recurrent miscarriage or implantation failure. The purpose of this guideline is to evaluate the role of immunomodulating therapy in ART. Unfortunately, many of the evaluated therapies lack robust evidence from well-designed adequately powered randomized controlled trials to support their use. Immunotherapies reviewed in the present document are either not associated with improved live-birth outcome in IVF or have been insufficiently studied to make definitive recommendations. (Fertil Steril® 2018;110:387–400. ©2018 by American Society for Reproductive Medicine.)

ORIGINAL ARTICLE

Immunosuppression with Tacrolimus Improved Reproductive Outcome of Women with Repeated Implantation Failure and Elevated Peripheral Blood Th1/Th2 Cell Ratios

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AJRI
American Journal of Reproductive Immunology

- prospective cohort study
- at the Sugiyama clinic
- November 2011 and October 2013.
- ≥5RIF patients(n = 42) with ↑ peripheral blood Th1 (IFN-γ)/ Th2 (IL-4) cell ratios
- 25 patients were treated with tacrolimus (treatment group) and 17 received no treatment (control group).
- Treatment group received tacrolimus 2 days before embryo transfer and continued until the day of the pregnancy test, for a total of 16 days.
- The daily dose of tacrolimus (1–3 mg) was determined based on the degree of the Th1/Th2 cell ratio.

Results

- clinical pregnancy rate :
  - treatment group was 64.0%
  - control group (0%)

which was significantly higher than that of the control group(P < 0.0001).

- miscarriage rate :
  - treatment group was 6.3%

- live birthrate :
  - treatment group was 60.0% (P < 0.0001).

- **There was no significant side-effect from tacrolimus in treatment group.**

- **No one developed obstetrical complications** during pregnancy.

- These results should be interpreted with caution as the study was subject to selection bias due to lack of randomization and a small sample size.

Conclusion

An immunosuppressive treatment using tacrolimus improved pregnancy outcome of repeated implantation failure patients with elevated Th1/Th2 ratios.

frontiers | Frontiers in Medicine

TYPE Mini Review
PUBLISHED 27 June 2023
DOI 10.3389/fmed.2023.1167876

OPEN ACCESS

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RECEIVED 16 February 2023

The impact of the use of immunosuppressive treatment after an embryo transfer in increasing the rate of live birth

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2020

if tacrolimus treatment for women who had experienced RIF was effective on their endometrium.

In patients with >3 RIF + ↑Th1/Th2 cells treatment with tacrolimus resulted in :

- ↑IL-10, LIF, IL-17
- ↓IFN-γ/IL-10, IFN-γ, IL-4.
- 40 % implantation rate
- 35 % live births rate.

2022

alterations in the populations of Th1 and 2 cells in the peripheral blood of RIF patients who were treated with tacrolimus, delivered a live born baby, and were in the course of pregnancy.

Two groups :

1) RIF without RPL.
2) RIF with RPL

The study found that in **both the groups**, tacrolimus treatment suppressed the Th1 immunity.

in the group with **RIF-plus-RPL**, the percentage of Th1 cells decreased **slowly** after the start of the treatment .

In **RIF patients**, tacrolimus was established to :

- ↑ expression of IL-10, LIF, IL-17
- ↓ expression of IFN-γ/IL-10, IFN-γ, and IL-4.
- Gradual ↓ in the numbers of Th1 cells.

group with high concentration of Th1 :

- lower ongoing pregnancy rate
- higher miscarriage rate

it is possible that an **increase in Th1 cells** can cause **pregnancy losses** and **implantation failures.**

As a result, it is recommended that tacrolimus treatment should not be stopped after the setting up of implantation of embryo in the mother.

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The Journal of Maternal-Fetal & Neonatal Medicine

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/ijmf20

Immunosuppressive therapy with tacrolimus is a potential drug candidate for the prevention of unexplained or preeclamptic stillbirths with Th1-dominant immune states: a case series of five patients

Michi Hisano, Koji Nakagawa, Tomo Suzuki, Rikikazu Sugiyama & Koushi Yamaguchi

To cite this article: Michi Hisano, Koji Nakagawa, Tomo Suzuki, Rikikazu Sugiyama & Koushi Yamaguchi (2023) Immunosuppressive therapy with tacrolimus is a potential drug candidate for the prevention of unexplained or preeclamptic stillbirths with Th1-dominant immune states: a case series of five patients, The Journal of Maternal-Fetal & Neonatal Medicine, 36:2, 2258254, DOI: 10.1080/14767058.2023.2258254

To link to this article: https://doi.org/10.1080/14767058.2023.2258254

- **five pregnant women** who:
  - had a **previous pregnancy history of unexplained or preeclamptic stillbirth.**
  - The patients had **infertility** associated with Th1/Th2 ≥10.3.
  - Th1 and Th2 cell and NK cell activities in peripheral blood were measured as clinical parameters during pregnancy.

- Tacrolimus treatment could:
  - correct the imbalance of Th1 and Th2 cells before implantation and during pregnancy
  - treating against implantation failures
  - preventing stillbirths.

Case 1–3:

- ❑ live births without pregnancy-related ~~complications~~.

In case 4:

- ❖ increased tacrolimus dose(2→ $3mg$)after a miscarriage 15W
- ❖ resulted in her first live birth
- ❖ she developed preeclampsia and severe FGR with ↑ Th1/Th2 **at 26 weeks** of gestation.

Case 5:

- ➢ had a previous history of early onset preeclampsia and HELLP syndrome, and an emergency cesarean section was needed for maternal safety **at 20 weeks** of gestation.
- ➢ The course of the next pregnancy was stable under tacrolimus treatment
- ➢ the HELLP syndrome recurred after preeclampsia **at 33 weeks** of gestation.
- ➢ Although an ~~imbalance in the Th1/Th2~~ cell ratio was not observed during pregnancy, **NK cell activity was markedly elevated before delivery.**

conclusion:

1) tacrolimus is a potential drug candidate for the **prevention** of unexplained or preeclamptic **stillbirth** with Th1-dominant immune states .

2) this experience with tacrolimus should be interpreted carefully because we could not compare placental findings before and after the intervention, which are important clues in investigating the cause of stillbirth.

3) novel biomarkers reflecting the maternalfetal immune status would be needed to determine the indications for tacrolimus treatment or the dose and duration of therapy in the future.

AJRI American Journal of Reproductive Immunology
Official Journal of the American Society for Reproductive Immunology

ORIGINAL ARTICLE

Obstetric and perinatal outcome of the women with repeated implantation failures or recurrent pregnancy losses who received pre- and post-conception tacrolimus treatment

Koji Nakagawa ✉, Joanne Kwak-Kim, Michi Hisano, Yoshimitsu Kasahara, Keiji Kuroda, Rikikazu Sugiyama, Koushi Yamaguchi

First published: 13 May 2019 | https://doi.org/10.1111/aji.13142 | Citations: 20

Method of study

- prospective observational study

- **109** women with RIF or RPL + ↑peripheral blood Th1/Th2 (IFN-γ+/IL-4) ≥10.3.
- All received tacrolimus before and during pregnancy (1-4 mg/d) and delivered a live-born infant(s).

1. Blood **concentrations** of tacrolimus were measured.
2. **Neuromotor development** of the babies was also evaluated.

Results

- Total **113** babies were born from **109** women, including **4 twin** pregnancies.

- **9** pregnancies including 4 twins were delivered **prematurely (8.3%).**
- **2** of 109 women showed obstetric complications, such as **hypertensive disorder** of pregnancy
- **1** baby (0.9%) had a **congenital abnormality**.

- Tacrolimus was detected in the maternal plasma, and its **concentration** did not significantly fluctuate during pregnancy while on daily administration regimen.

- There were **no differences** in babies' birthweight, placental weight, and lymphocyte proportion (%) of the umbilical cord among the women **with different tacrolimus dosing**.

- ***Neuromotor development*** of the babies exposed to tacrolimus in utero **was comparable** with that of babies from the general population**.**

Conclusion

- tacrolimus treatment for women with RIF and RPL was not associated with obstetrical and perinatal complications.

- A large size study is needed to confirm this finding.


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